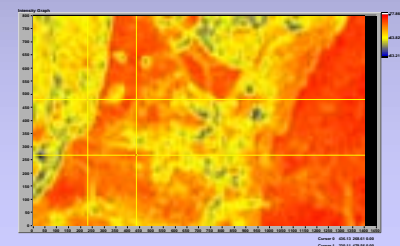


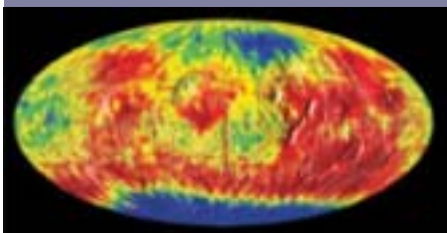
Life Detection and Sensors

Bio-Info-Nano Science

Michael C. Storrie-Lombardi, M.D.



Raman Spectroscopy & Native Fluorescence Imaging Laboratory
 Center for Life Detection & Virtual Planetary Laboratory
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Core Projects

In Situ Biosignatures

- **Antarctic Cryptoendolithic Communities** (H. Sun, G. McDonald, JPL)
- **Deep Sub-ocean Vesicular Basalts** (M. Fisk, OSU; S. Douglas, JPL))
- **Evaporite Crystals (Halophiles)** (M. Mormile, UMR)
- **Yellowstone Hydrothermal Biofilms** (A. Neal, MSU)
- **Hydrothermal Vents and Arctic Ice** (A. Lane, JPL)
- **Paleobiology-Fossil Ferns/Cyanobacteria** (A. Czaja, L. A. Smith, UCLA)

Remote and In Situ Biosignature Detection

- **Entropy Image Analysis/Neural Networks** (R. Bhartia, JPL)
- **Virtual Planetary Laboratory** (V. Meadows, JPL/SIRTF)
- **Soap Lake Microbial Observatory** (H. C. Pinkart, CWU)
- **Serpentinization/Habitable Planets** (K. Nealson, R. Rye, USC)
- **Stromatolites/Complexity Analysis** (F. Corsetti, G. Tinetti, USC)
- **THEMIS** (K. Nealson, G. Tinetti, USC)

Instrument Development

(Industrial Collaborator: W. Hug, Photon Systems, Inc.)

- **Laboratory Deep UV Raman Spectrometer & Fluorescence Imaging System**
- **Field Deep UV Raman and Fluorescence Organic Detector**
- **Mars Ultraviolet Raman Fluorescence Explorer (MURFE)**

Biology, Information, and Nano- Technology and Science

- NASA Ames Research Center and USRA jointly sponsored a workshop titled "Biology-Information Science-Nanotechnology Fusion & NASA Missions," October 7-9, 2002 at the Ames Research Center. Co-chairs included G. Scott Hubbard (NASA), T.R. Govindan (NASA), Lewis Peach (USRA) and Kathleen Connell (USRA) <http://binfusion.arc.nasa.gov>
- The workshop discussed the science challenges generated by NASA missions and the possible advantages of cross-disciplinary research in biology, information science, and nano-technology.
- The material included here was presented to outline two science problems that might serve as drivers to encourage such an interdisciplinary effort.

NASA Astrobiology Institute Overview

- *Since only 5 members of the NAI attended this meeting and since many of the participants seemed unaware of the distributed and inter-disciplinary model developed by the NAI over the last five years, a series of slides were presented outlining the NAI Roadmap, Lead Teams, Field Sites, Multidisciplinary Science, and NASA Mission Involvement.*
- *These are not reproduced here, since they are well known to all of us in the NAI community.*

NAI Efforts in In Situ and Remote Biosignature Detection: Needs for Both Nano-technology and Information Science

In Situ (including ISS as an extreme ecological niche)

1. ER Triage as a Model for In Situ Exploration:

Mass, Power, and Volume Constraints for 30 devices

2. Distributed surveys: 1000 sensors across Mars

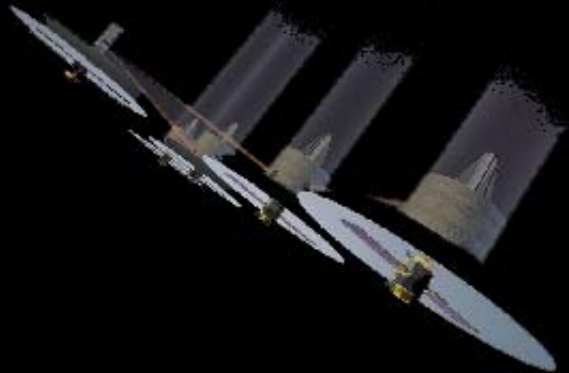
Remote Sensing

Astronomical detection of biosignatures from extrasolar planets requiring nano-scale geobiology information

The Virtual Planetary Laboratory: Characterizing Extrasolar Planets

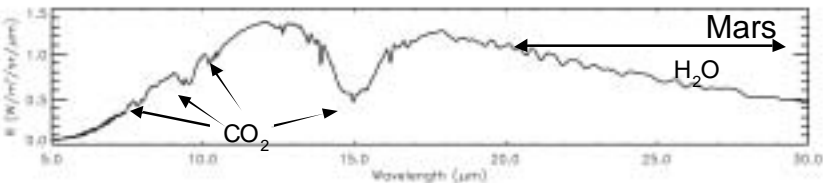
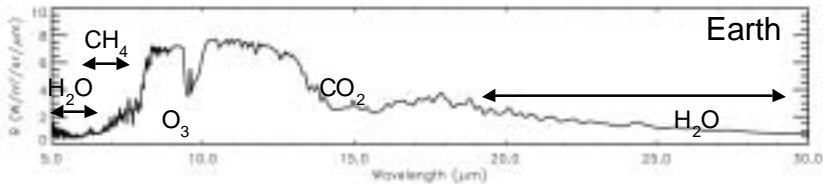
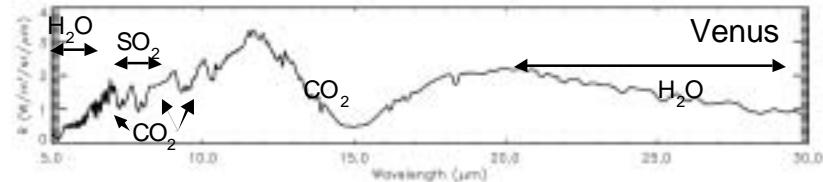
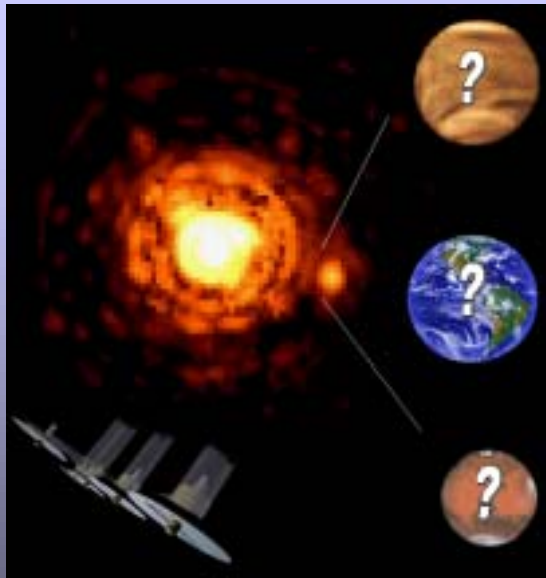


**Using Nano-scale Inputs for Remote Detection of
Complex Geobiological Systems...**

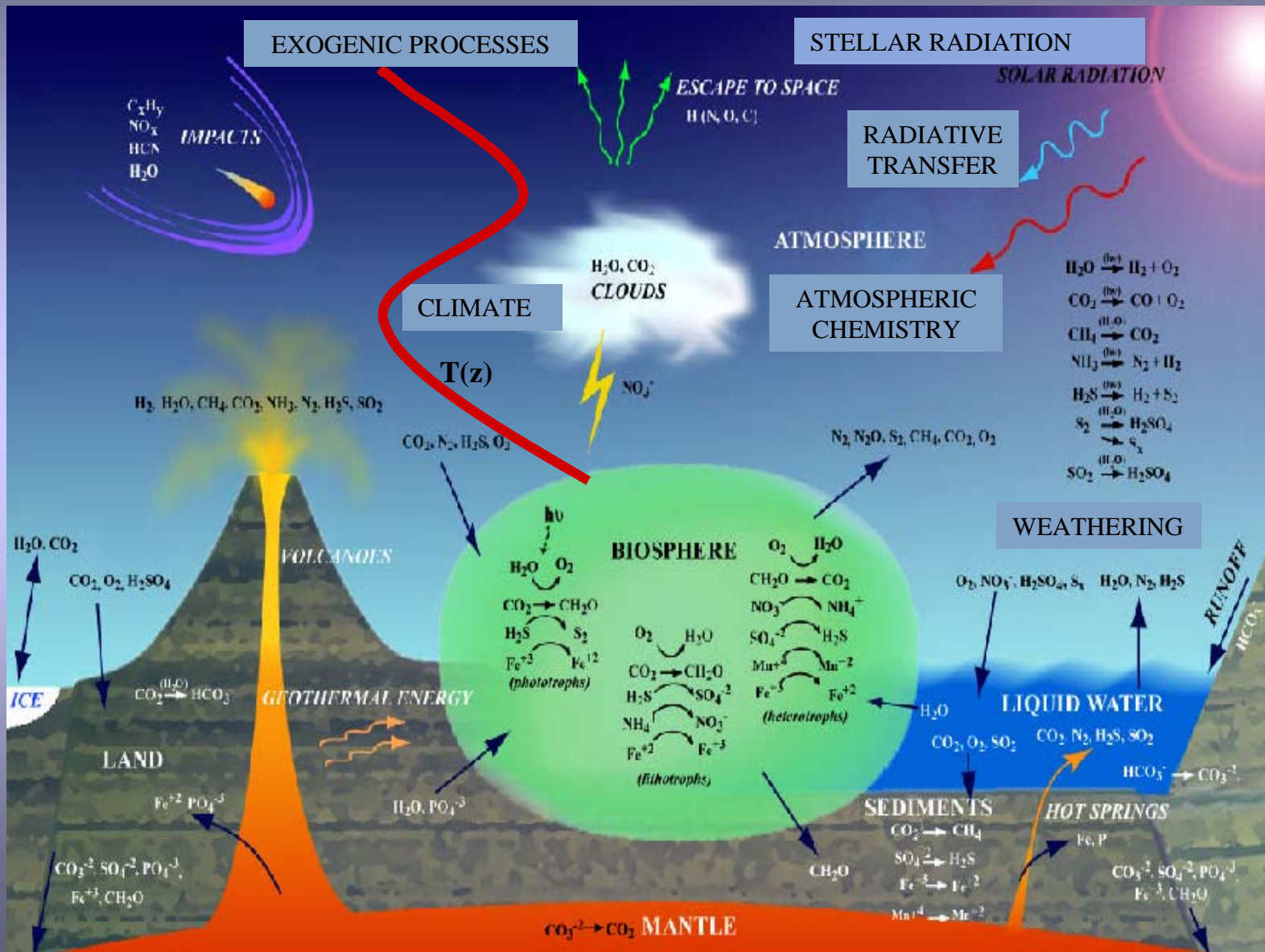


VPL RESEARCH GOALS

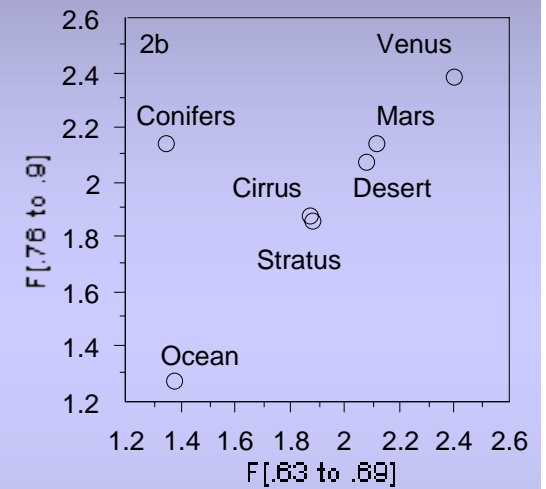
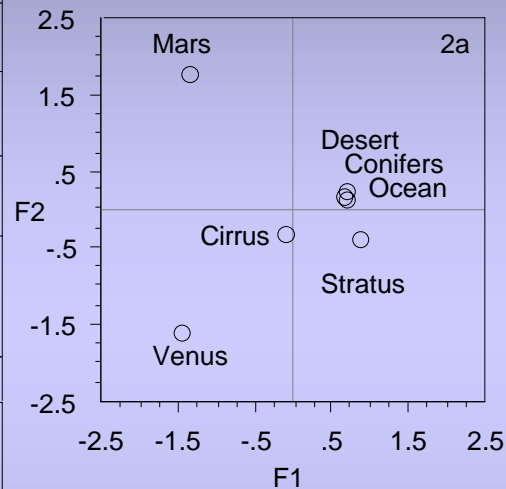
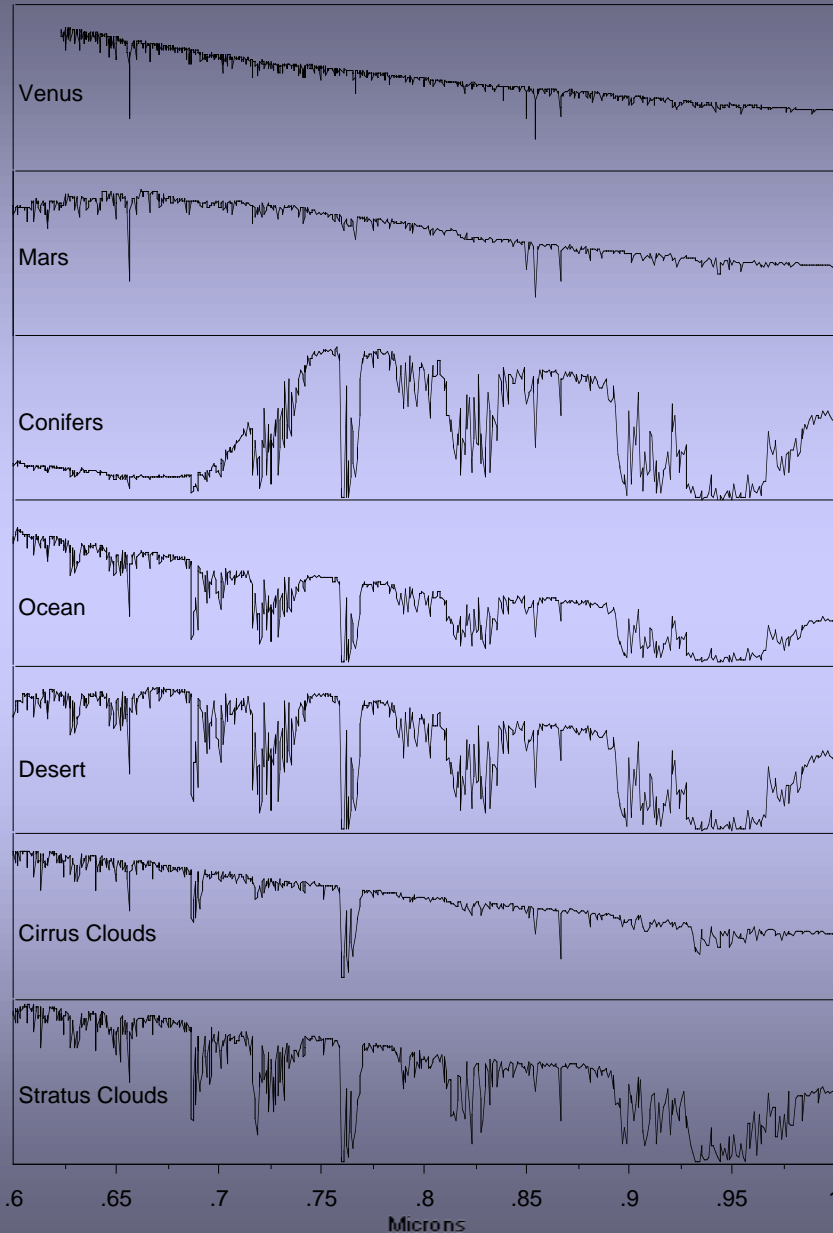
- ☾ To understand the plausible range of atmospheric and surface compositions for terrestrial planets, and
- 🌍 To learn how to use spectra to discriminate between extrasolar planets with and without life.
- ≈ The results will drive the design and search strategies for future planet detection and characterization missions.



SIMULATING PLANETS AND THEIR SPECTRA



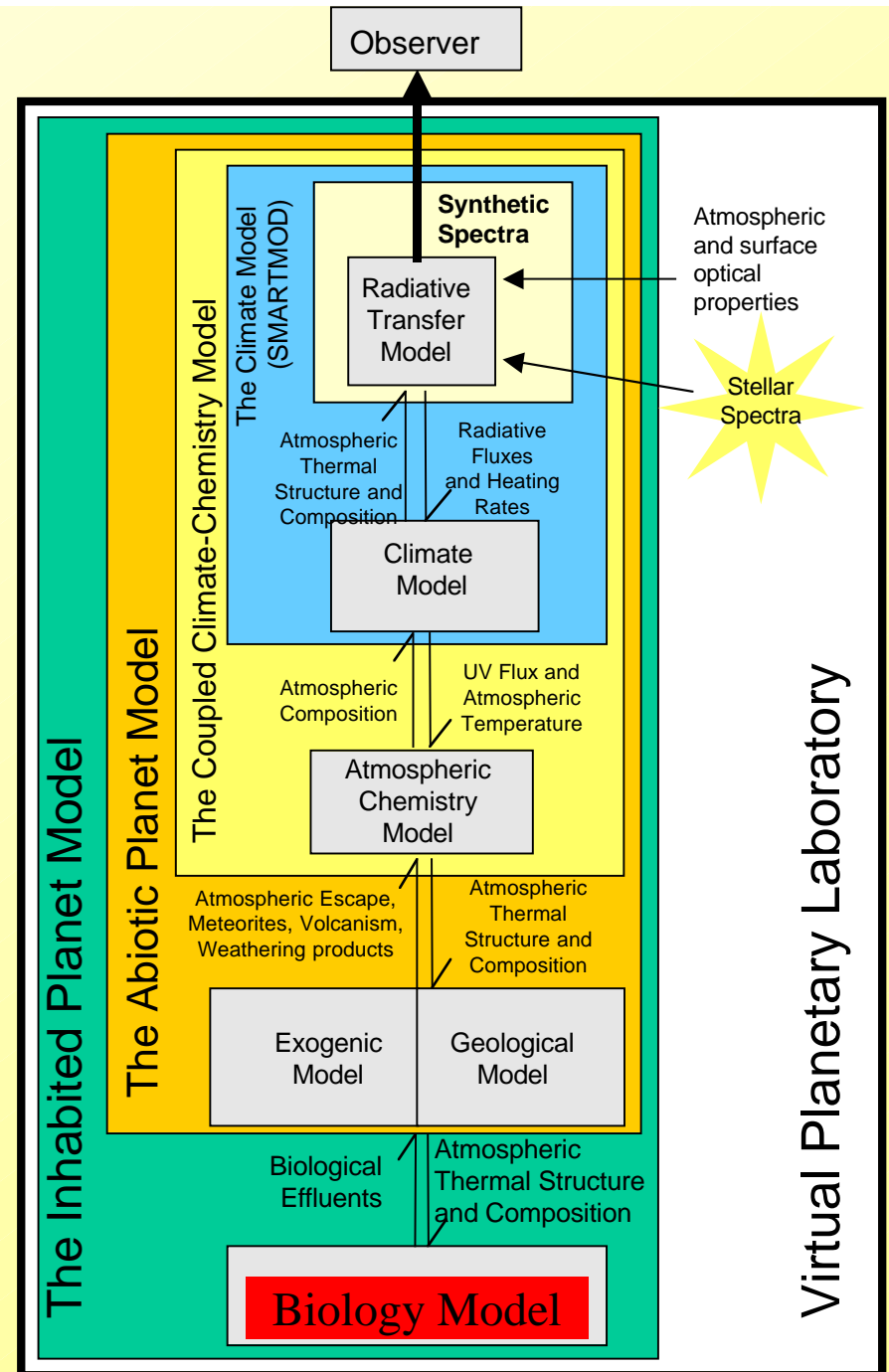
Spectral Classification of Planets



Many spectral types are possible in theory. So how do we constrain our search?

THE VIRTUAL PLANETARY LABORATORY

- Constructed with six interlocked models.
- Each model contributes to a range of synthetic spectra to identify habitable planets or potential biosignatures, and to derive astronomical instrumentation requirements.
- The VPL must:
 - improve modeling methods, inputs and computational efficiency.
 - cover a broad range of wavelengths
 - consider planets other than Earth, around stars other than our Sun.
 - include non-oxygen producing life
 - ultimately provide a comprehensive, *flexible* tool which can be used by a broader community.



Extreme Environments and Nanometer to Planetary Scale Biosignatures

Remote Sensing: *Ground Truth and Detection Sensitivity*

**Ecosystem Complexity: *Analytic Solution and
Experimental Data for Complex Feedback Systems Between
Organisms & Environment***

**Long Range Stability of a Biology Driven Environment:
*A 10 Year Search in a 10 Gy Search Space***

Overview of Work by NAI at JPL Sites

DATA SET EXAMPLES

- Alkaline Lakes {Microbial Observatory}
- Antarctic Cryptoendolithic Communities
- Deep Subocean Vesicular Basalts
- Evaoprite Crystals (Halophiles)
- Paleobiology-Fossil Ferns/Cyanobacteria
- Ultramafic Environments

COLLEAGUES

- (H. C. Pinkart, CWU)
- (H. Sun, G. McDonald, JPL)
- (M. Fisk, OSU; S. Douglas, JPL)
- (M. Mormile, UMR)
- (A. Czaja, L. A. Smith, UCLA)
- (K. Nealson, R. Rye, USC)

MULTI-PROBE INSTRUMENTS

- Needs
- Volume, Mass, Energy Constraints
- Example: Fluorescence and Rayleigh Imaging
+ Raman Spectra

(W. Hug, Photon Systems, Inc.)

- VPL INPUTS
- Entropy Image Analysis
- Stromatolites/Complexity Analysis
- Nano-scale geology and macro-scale biosignatures

(V. Meadows, JPL/SIRTF)

(R. Bhartia, JPL)

(F. Corsetti, G. Tinetti, USC)

Mono Lake



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Mono Lake Tufa



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Soap Lake, Washington



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Soap Lake Microbial Mat Community



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Soap Lake Microbe Gas Production



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Soap Lake Layered Microbial Community



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The Cedars



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The Earth's Mantle



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High West Site 1



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Little Faithful



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Mixed Microbial Mat on Carbonate



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“Carbonate Falls”



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Old Stromatolite

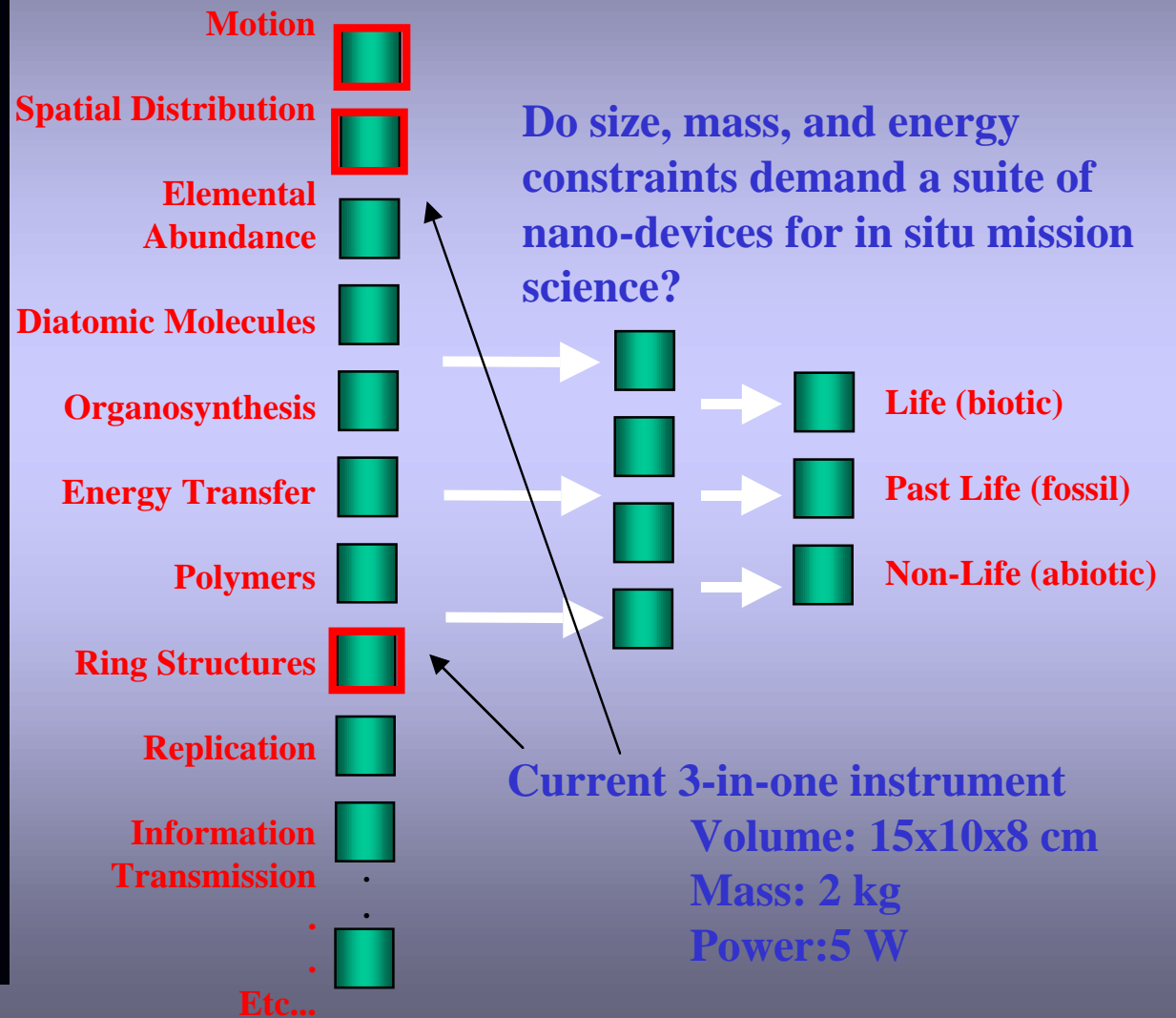
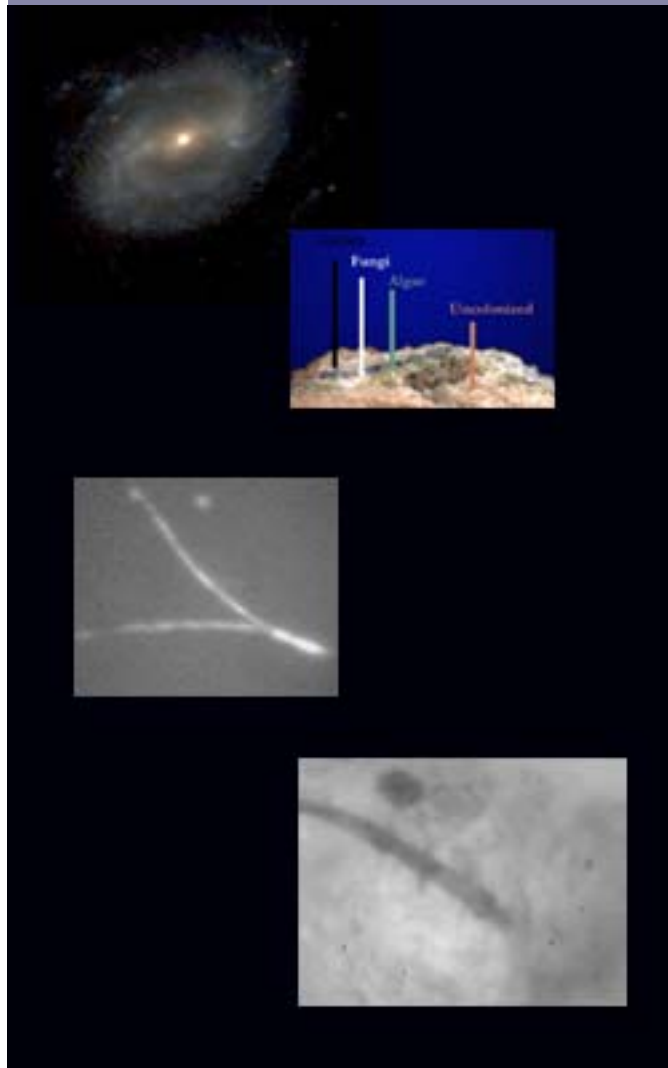


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So what do we measure and in how many ways?

Multi-Sensor Probabilistic Life Detection

Fundamental Event → Neural Network → Bayesian Classification



UV Raman Spectroscopy & Native Fluorescence Imaging

UVRS I



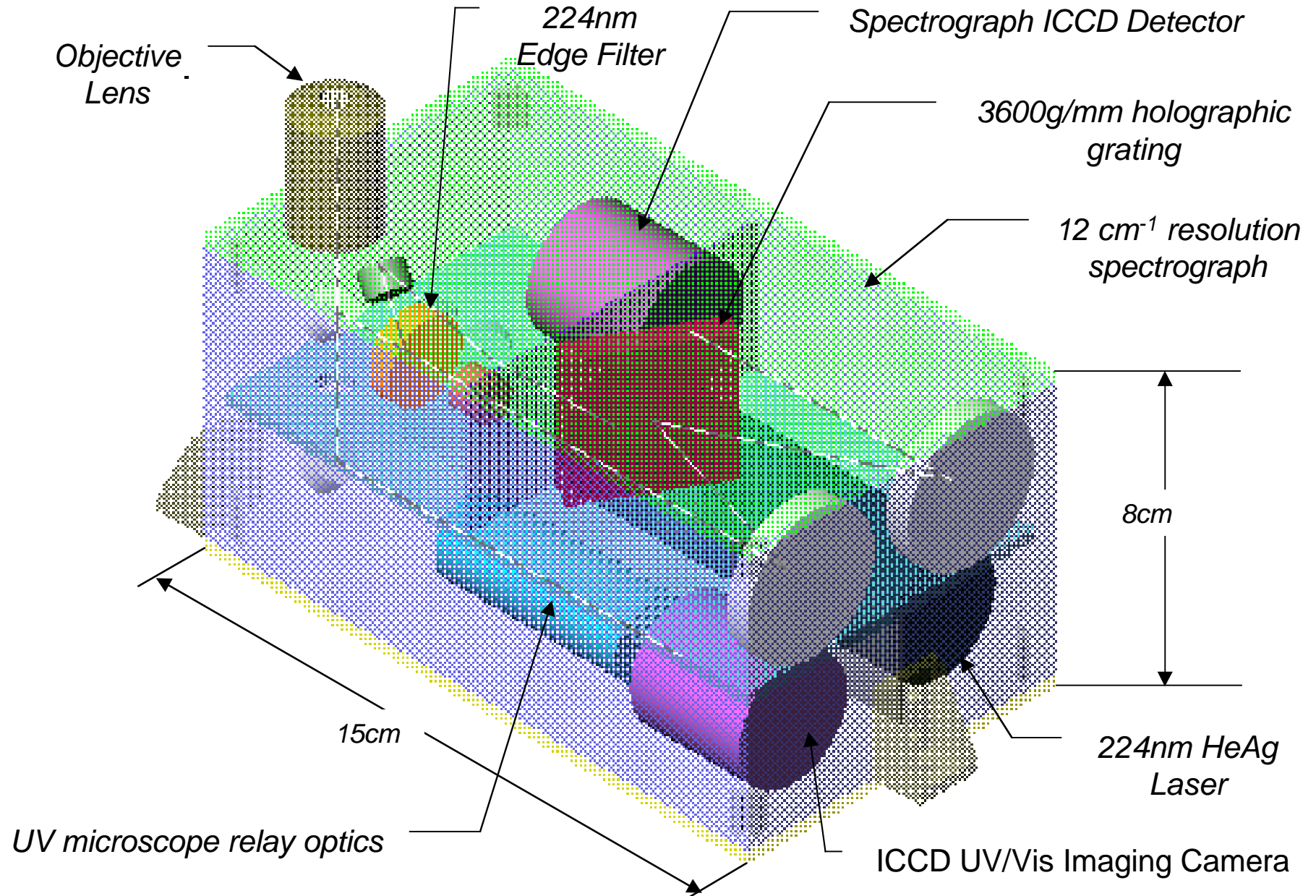
UVRS II



Figure 1a depicts the first Ultraviolet Raman Spectrometer (UVRS I) and native fluorescence imaging system constructed at the Jet Propulsion Laboratory Center for Life Detection. Although this first system requires less than half the mass, power, and volume of its predecessors, it still weighs 150 kg, occupies 4 m³ and requires ~500W. Figure 1b depicts the recently developed portable version of the system (UVRS II) weighing 10 kg (plus laptop), 20cm x 25cm x 50.8cm, and drawing <100W. Both systems operate at 224nm, 248nm, or 325nm. The system can be easily configured to operate in a variety of extreme environments. The two systems were developed in a joint effort between Center for Life Detection and Photon Systems, Inc., Azusa, CA. The systems provide three (3) data sets:

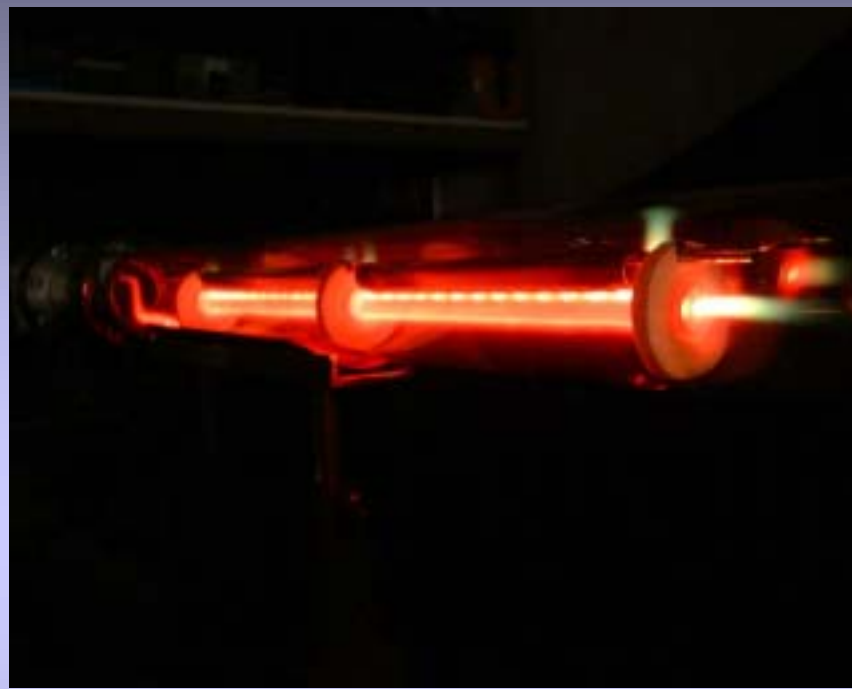
Multi-bandpass Microscopic Imaging UV to far Red
Fluorescence Multi-bandpass Imaging
Raman and Resonance Raman Spectra

Mars Ultraviolet Raman and Fluorescence Explorer (MURFE)



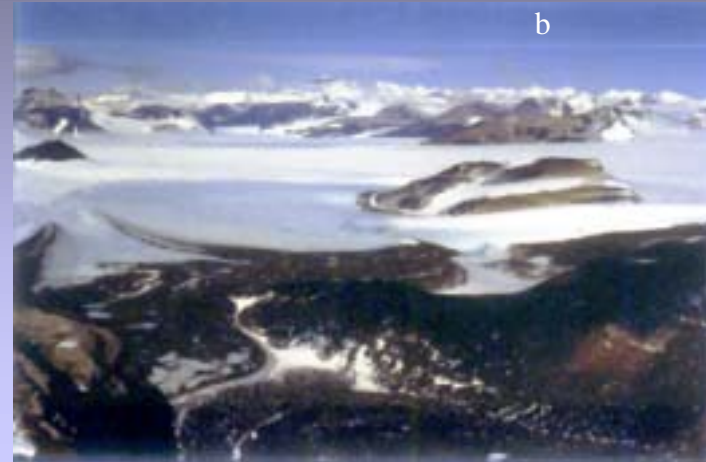
Center for Life Detection Rover-compatible *in situ* instrument for generating deep UV fluorescent images and Raman spectra during a Mars surface or sub-surface mission with mass ~2kg and power consumption < 5W.

HeAg and NeCu Lasers



- *Output >100mW at 224nm or 248nm*
- *Instantaneous startup (no preheat or standby)*
- *Narrow linewidth (<3GHz or 0.1cm⁻¹)*
- *Size weight and power consumption of HeNe laser*

Exploring Cryptoendolithic Communities with Deep UV Native Fluorescence and Raman Spectra in The Antarctic Dry Valleys



Antarctica (Figure 2a) contains a dry desert region (2b) with sandstone rocks (2c) serving as refuge sites for complex microbial communities. These cryptoendolithic communities, their survival strategy in a cold, dry, high UV flux environment, and their interaction with the surrounding rock matrix provide an accessible Mars analog ecosystem. *Images courtesy of H. Sun.*

Antarctic Dry Valleys Cryptoendolithic Community

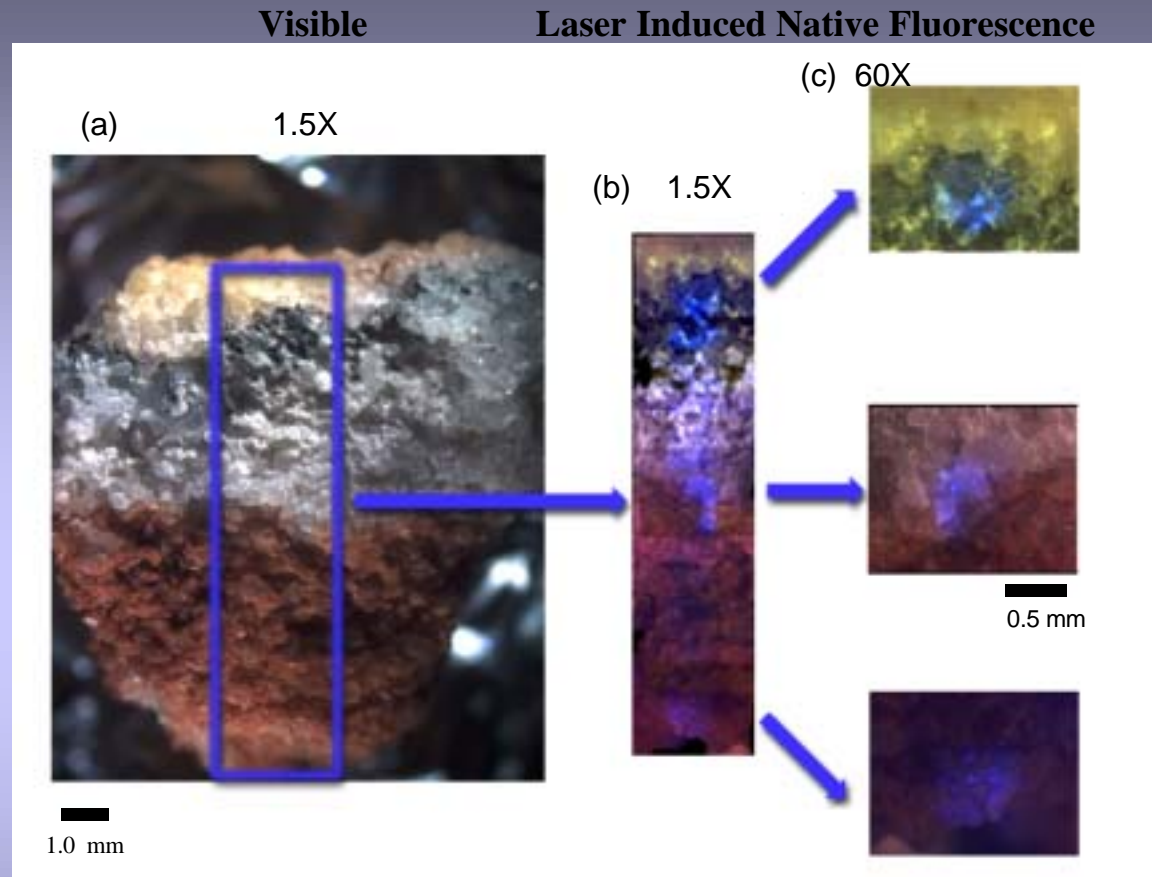
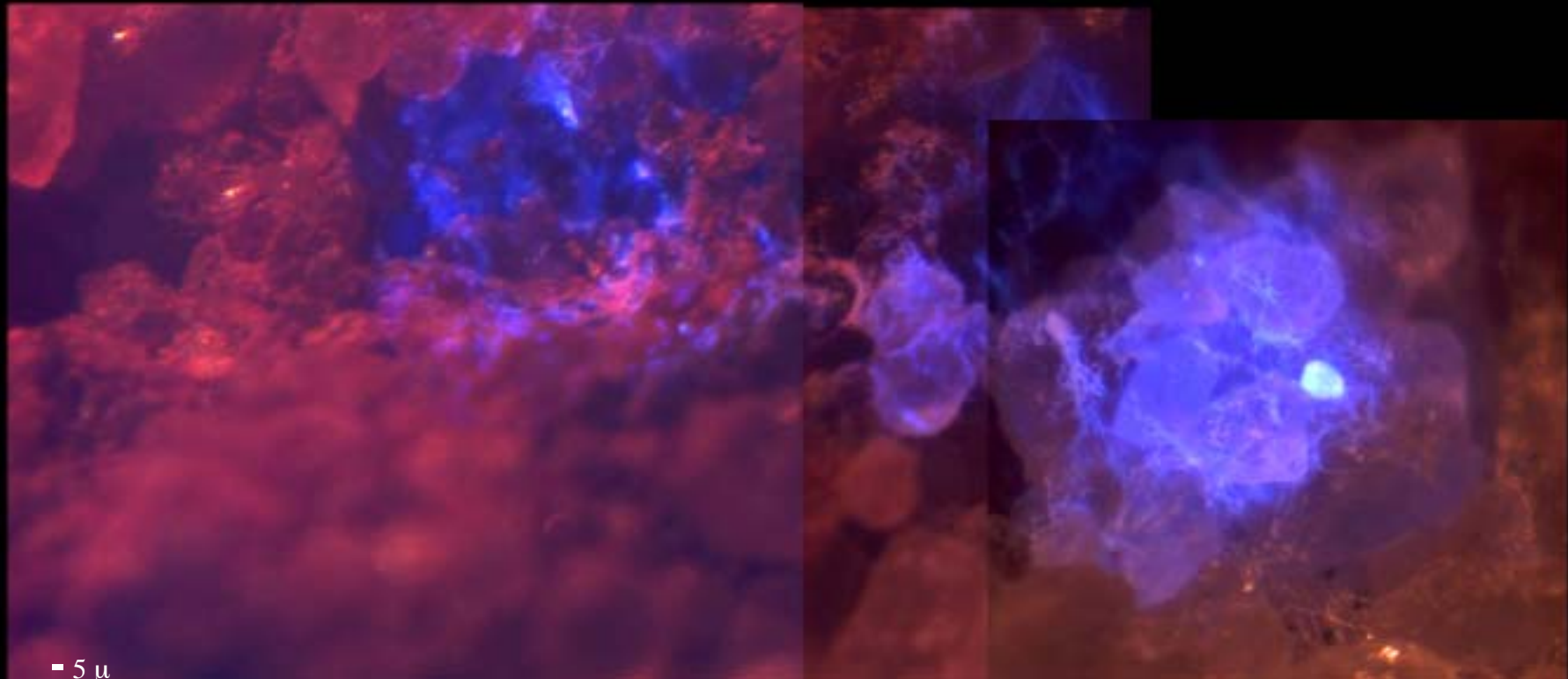


Figure a is a low resolution (1.5X) image overview of a 10 x 10 mm internal face of a cleaved sample of Antarctic Dry Valleys sandstone (sample courtesy of E. Friedman). Illumination in broadband visible light reveals a characteristic biosignature for cryptoendolithic communities: the stratified layering just below the mineral crust. In Figure b excitation using a 224 nm deep UV laser for illumination reveals a lightening-bolt of native fluorescence heralding a complex community of microorganisms extending some 8.5 mm into the interior of the rock. In Figure c optical magnification to 60X and illumination with both broadband visible light and 224nm UV laser source reveals fine structure details of the microbial community. The 224 nm wavelength induces native fluorescence activity in aromatic amino acids found in all microbial life on this planet.

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Antarctic Cryptoendolithic Communities: 224 nm Excitation of Native Fluorescence in Sample AL845-504



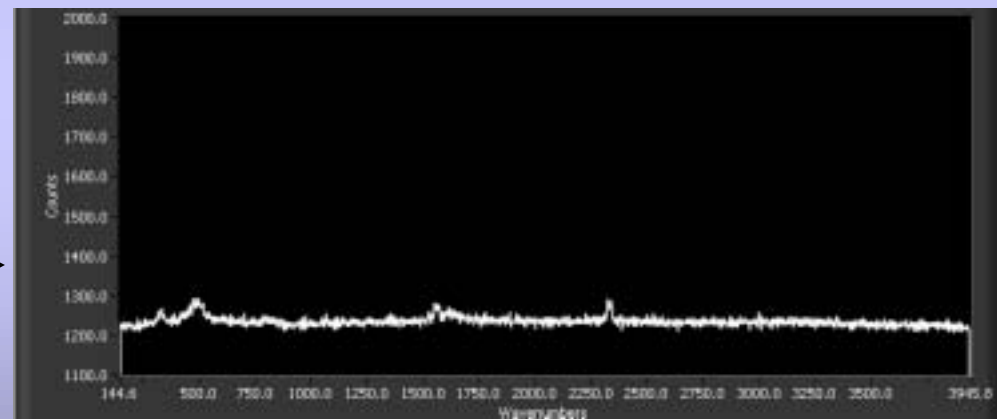
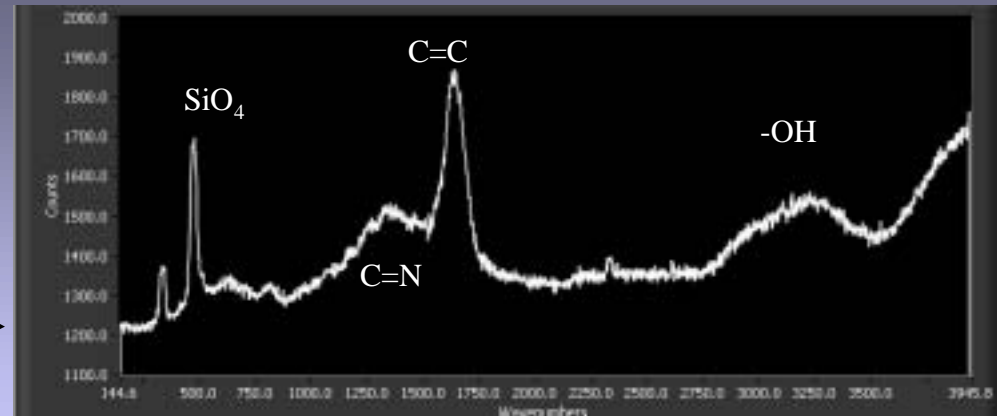
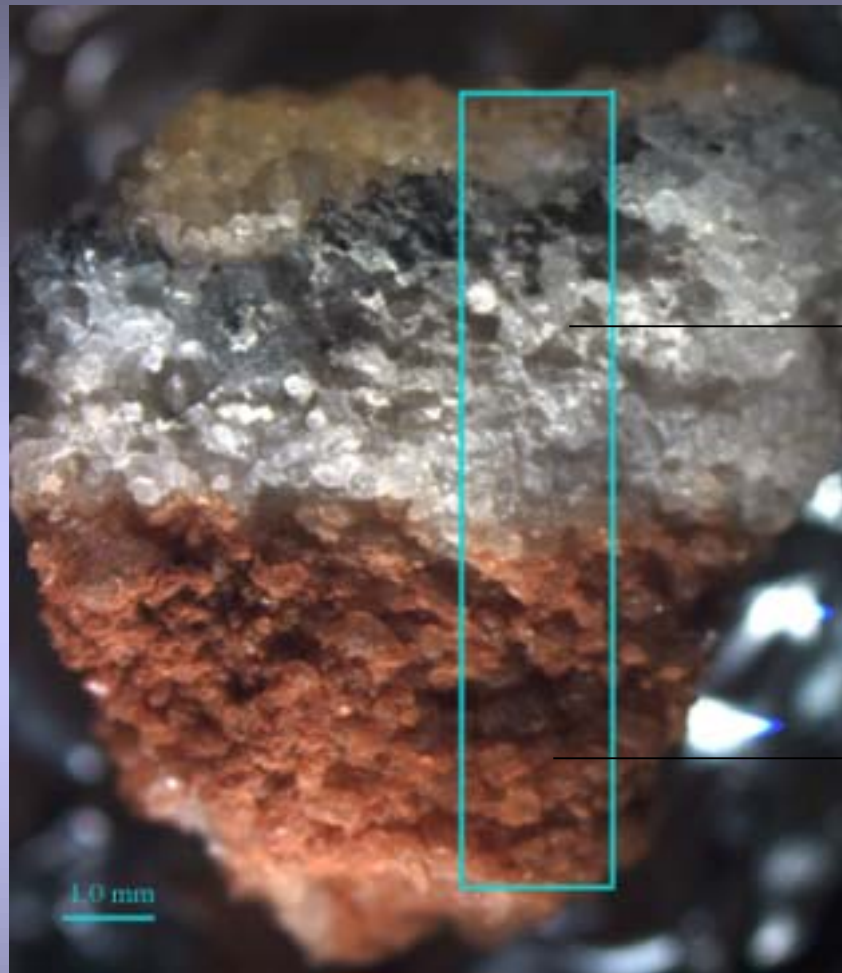
60X Magnification

<- Red White -> Rock Surface ->

224 nm laser excitation of an Antarctic rock cryptoendolithic community elicits a fluorescent response from filamentous strands extending across a red|white mineral boundary zone. The excitation wavelength falls within the fluorescence absorption bands for the aromatic amino acids. The filaments are most likely a previously identified fungal community. Many filaments in the red(iron-rich) zone are covered with red granules morphologically similar to grains of hematite. Microbial life in this extreme environment lives in close association with the mineral matrix of the surrounding sandstone. Co-registration of visible and native fluorescence images allow a detailed examination of the 3-dimensional organic-inorganic interaction. {Specimen courtesy of E. I. Friedman & H. Sun}

For more information contact M. C. Storrie-Lombardi, M.D. mcs1@jpl.nasa.gov

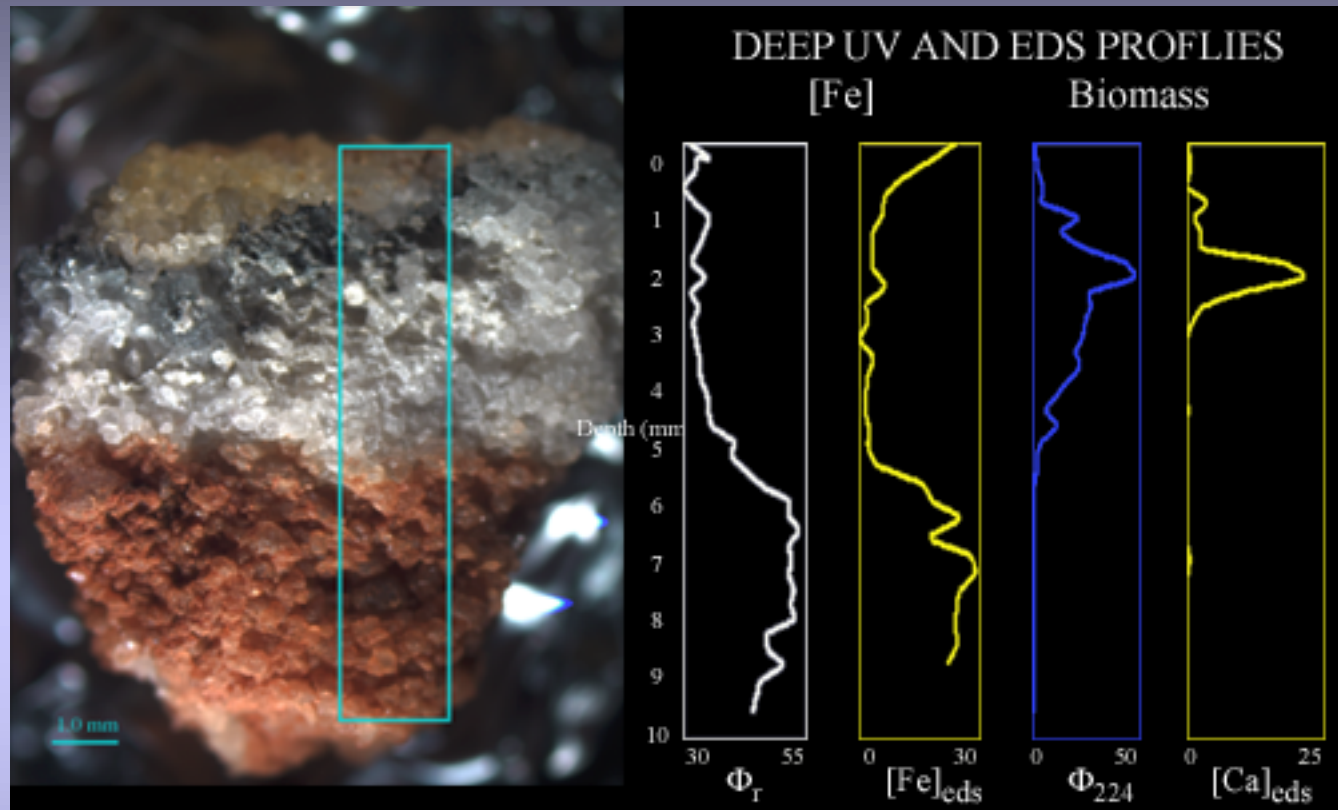
Deep UV Resonance Raman Spectra of Antarctic Sandstone



Deep UV resonance Raman spectra elicited with a 1 second exposure to 10 μ W of UV light from a 224.3 nm hollow cathode ion laser in the Fe-depleted (a) and Fe-rich(b) layers. The resonance effect enhances the Raman response by $\sim 10^6$. Iron acts as an efficient UV absorber, a trait exploited by microbial communities such as this one. Both the excitation and Raman shift photons can be absorbed since the wavelength shift occurs in less than 10 nm. As a result the signal in the Fe-rich layer exhibits more than an order of magnitude reduction in signal strength.

For more information contact M. C. Storrle-Lombardi, M.D. mcs1@jpl.nasa.gov

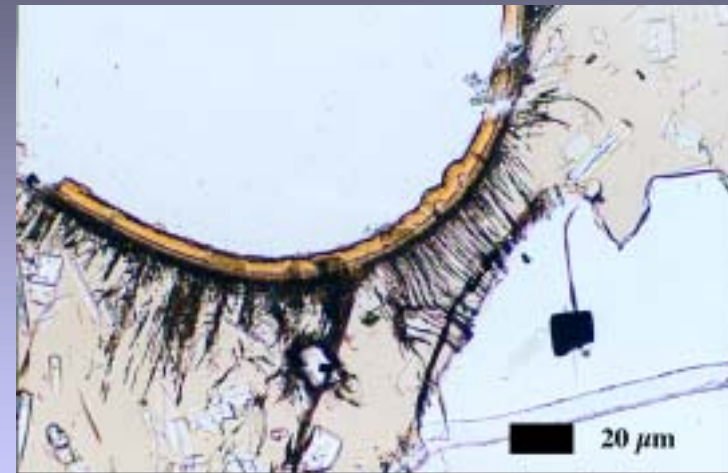
Iron and Biomass Profiles of a Cryptoendolithic Community



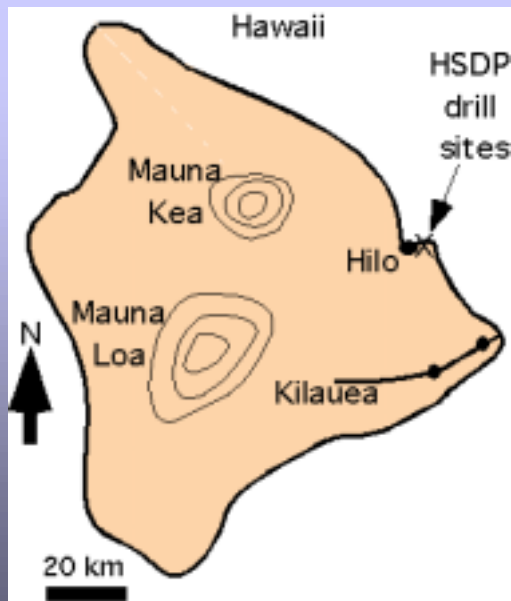
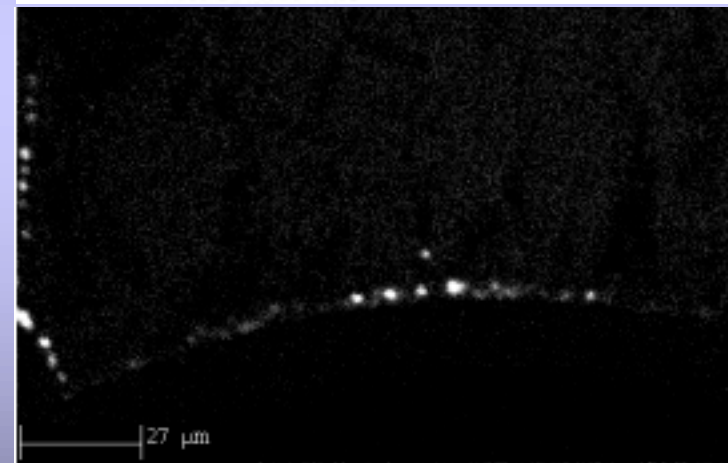
Estimation of the iron concentration using energy dispersive x-ray spectroscopy (EDS) produces an $[Fe]_{eds}$ profile across an Antarctic sandstone cryptoendolithic community. The estimation of $[Fe]$ using total red-band flux (Φ_r) normalized for total RGB flux during white light illumination produces a markedly similar profile. Significant deviation occurs only in the upper 1.0 mm of crustal material. This color/EDS difference is most likely due to the fact that the crustal Fe fraction is present as a hydrated amorphous form, while in the lower red zone it is present as hematite. Estimates of biomass load by normalized blue-band flux (Φ_{224}) results in a profile markedly similar to that produced by EDS measurement of calcium concentration $[Ca]_{eds}$.

For more information contact M. C. Storrie-Lombardi, M.D. mcs1@jpl.nasa.gov

Evidence of Biological Activity in Hawaiian Subsurface Basalt

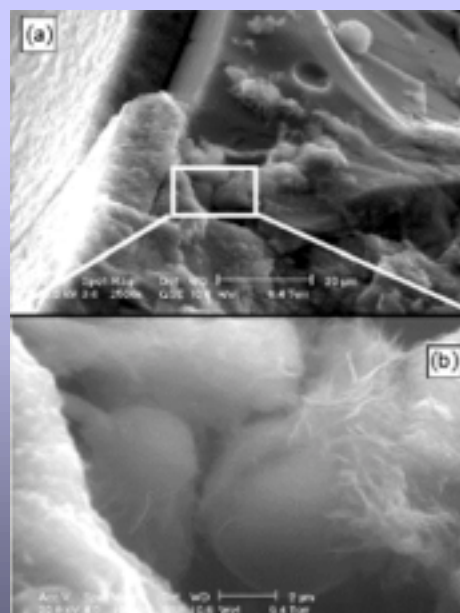
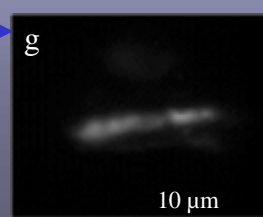
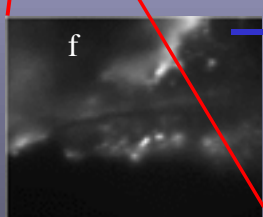
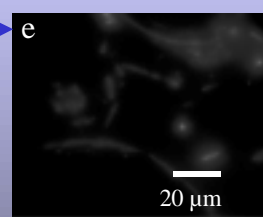
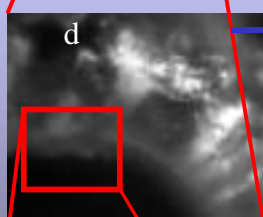
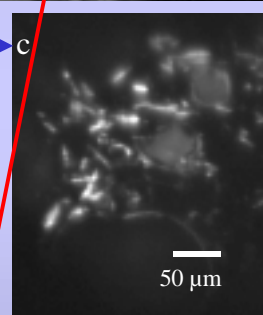
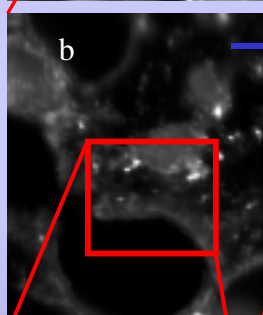
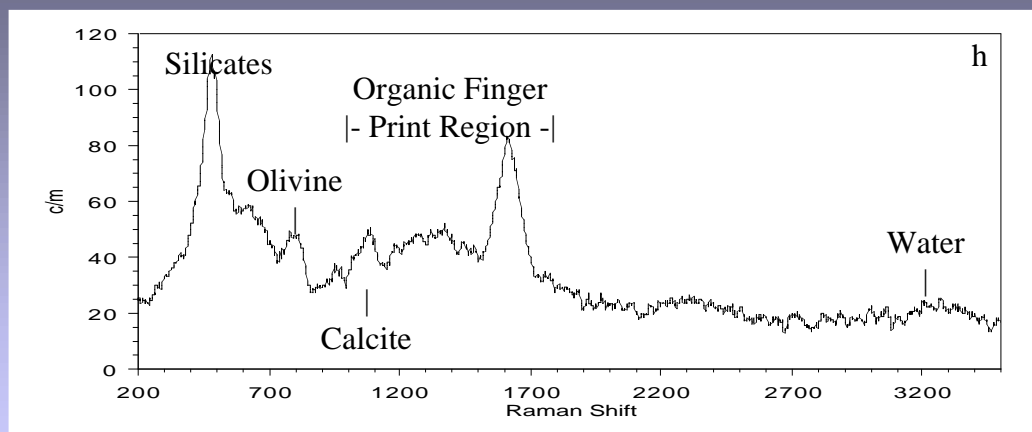
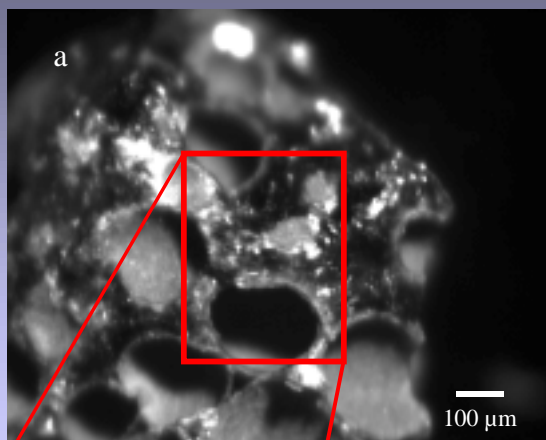


HSDP R531 9.5 1354 mbsl



Samples of subsurface (1.3 km) basalt obtained by the Hawaii Deep Core Drilling project exhibit vesicles containing clay and accumulations of phosphorous at the clay-basalt interface. Petrographic image and X-ray map by M. Fisk, Oregon State University

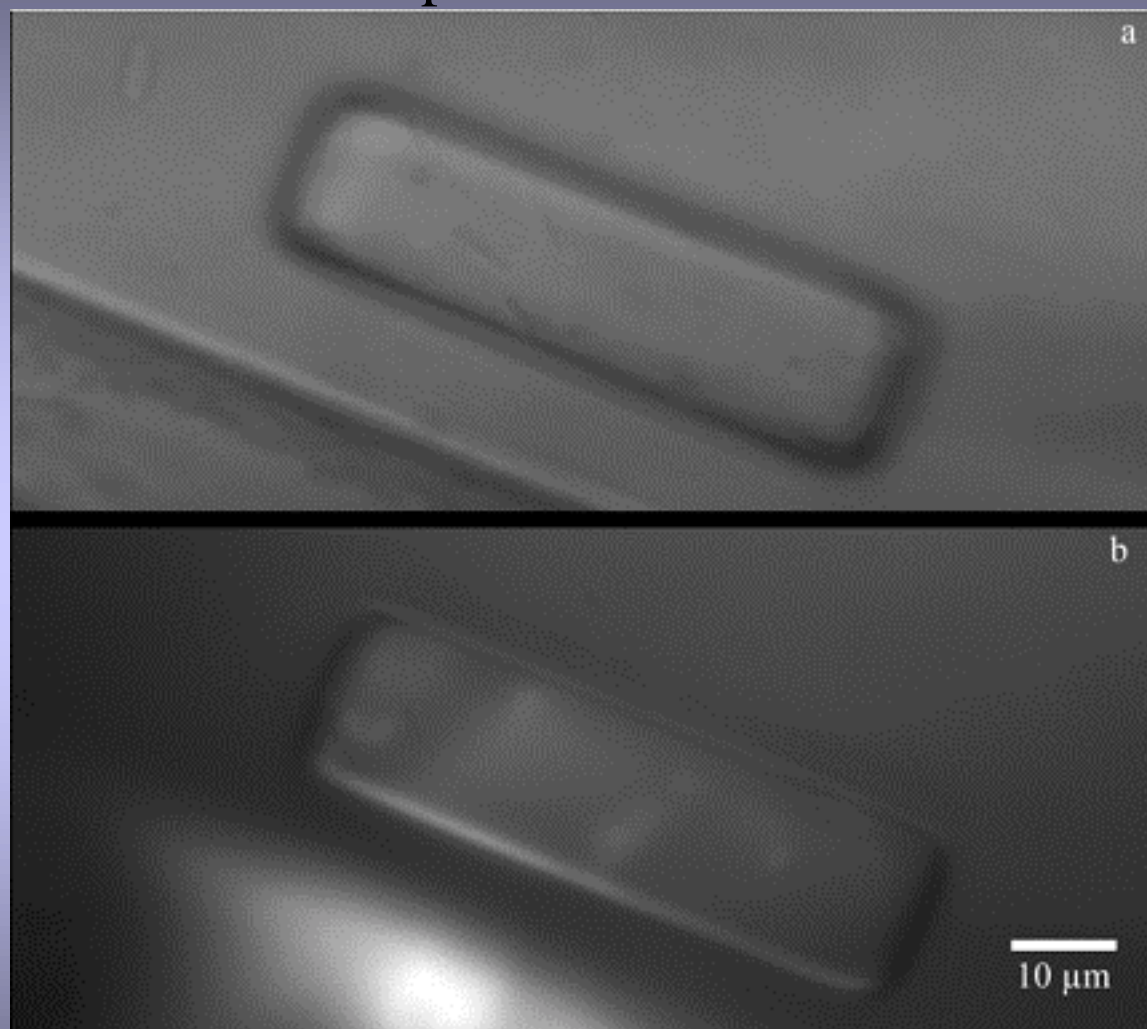
Deep UV Fluorescence Images and Resonance Raman Spectra of Vesicular Basalt



Visible reflectance (Figures a, b, d, and f) and fluorescent (Figures c, e, and g) images of deep subsurface volcanic basalt reveal an area of native fluorescence at the clay-mineral boundary of two vesicles. The native fluorescent images were a result of 224 nm deep UV excitation using a hollow cathode ion laser (Photon Systems, Inc.). Resonance Raman data (Figure h) obtained with 248 nm laser excitation reveal activity between 1300 and 1600 wavenumbers. In terrestrial samples such resonance activity usually indicates the presence of nucleic and aromatic amino acids. Environmental scanning electron microscopy (S. Douglas, CLD) subsequently revealed the presence of microbial colonies just below the clay-basalt interface. These 1354 meter subsurface samples of Muana Kea basalt were courtesy of M. Fisk.

For more information contact M. C. Storrie-Lombardi, M.D. mcsll@jpl.nasa.gov

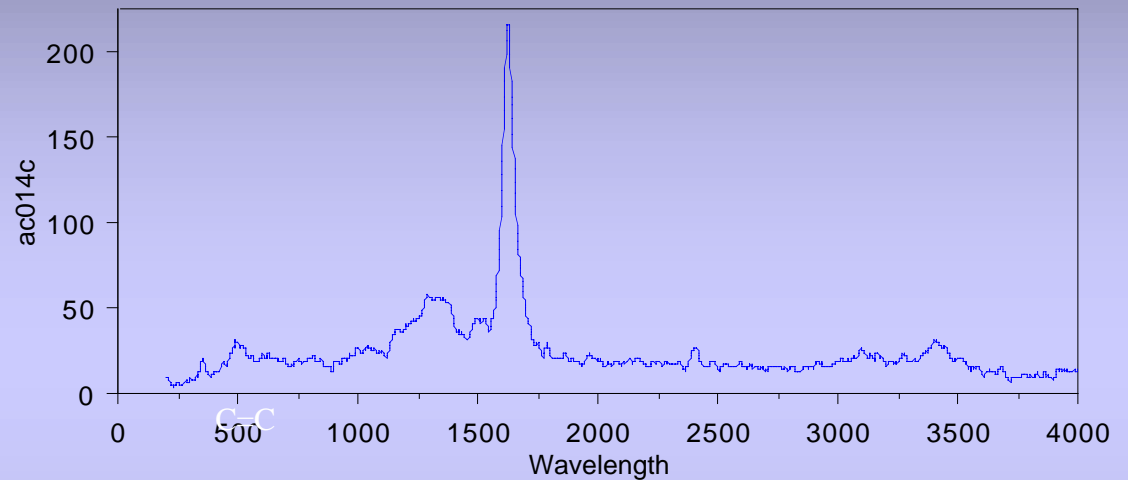
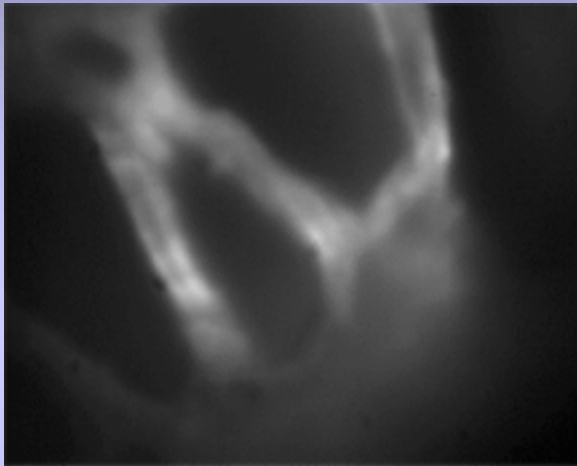
Deep UV Fluorescence Images of Halophilic Microorganisms in Evaporite Inclusions



Visible light (Fig a) and laser-induced native fluorescence images of halophilic bacteria in a halite crystal. Native fluorescence was induced with 224.3 nm excitation. Fluorescent images appear blurred because the bacteria were in rapid motion due to either convection currents or flagella activation. *Samples courtesy of M. Mormile.*

For more information contact M. C. Storrie-Lombardi, M.D. mcs1@jpl.nasa.gov

Deep UV Fluorescence Images and Raman Spectra of Fossil Ferns



C=N

-OH

48 million year old fossil ferns exhibit a markedly well-preserved deep UV resonance Raman spectra. Excitation was at 248.6 nm. Total time of UV exposure was 1 second with <100 microwatts delivered to sample. *Samples courtesy of A. Czaja, UCLA.*

Deep UV Native Fluorescence in Counter Bioterrorism

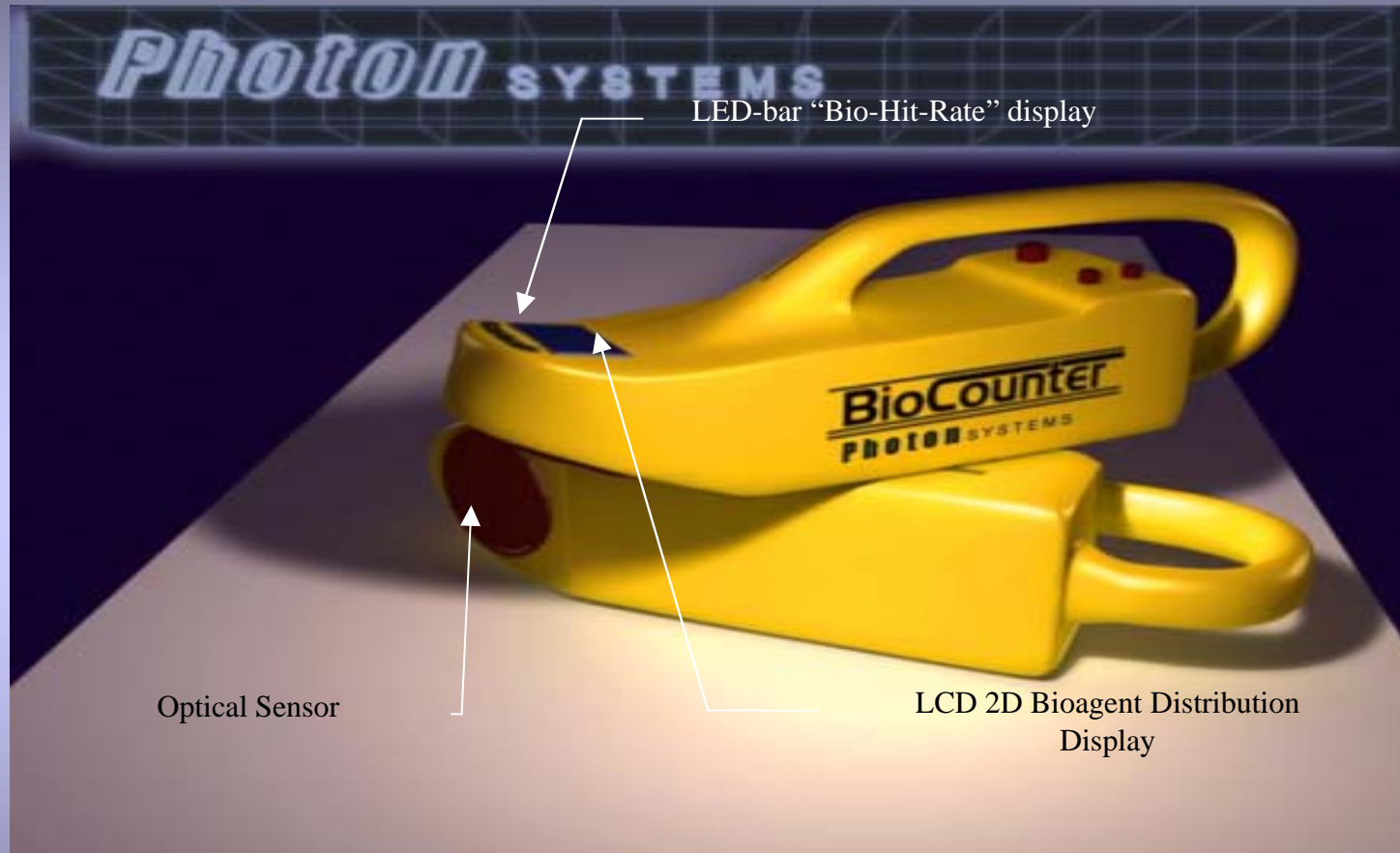
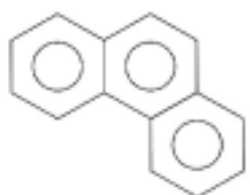


Illustration of two “BioCounter” Biological Agent Surface Sensor (BASS) instruments. Two views are shown to illustrate the top with LCD and LED-bar displays, and bottom with the sensor lens that focuses the raster scanned deep UV laser beam and collects fluorescence emission. The lens is pointed down to facilitate searching of horizontal surfaces while wearing cumbersome biohazard suits.

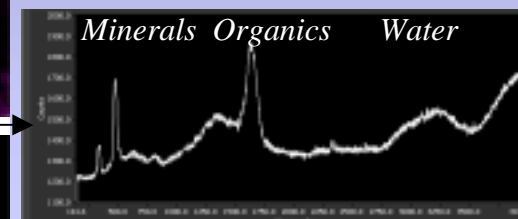
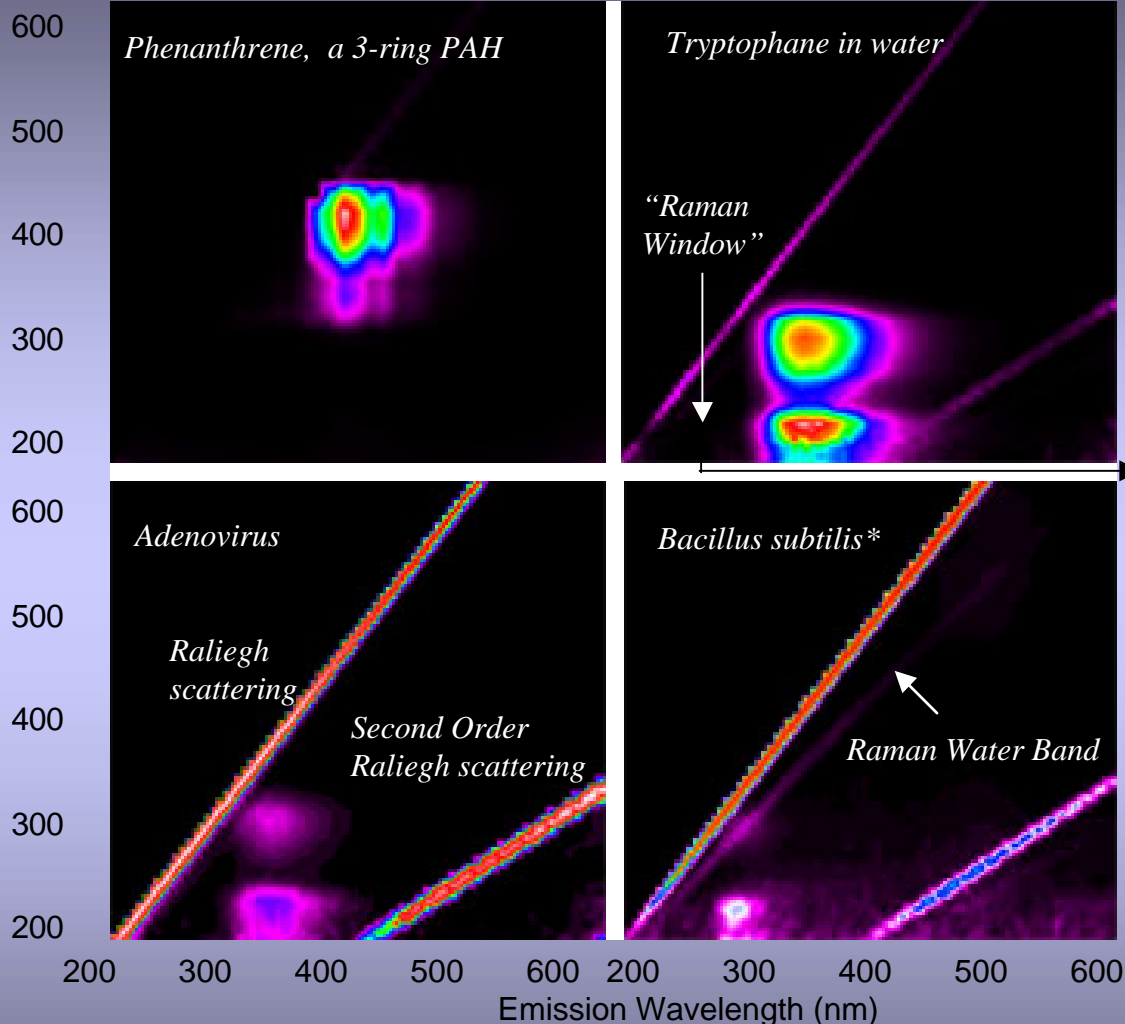
For more information contact W. Hug, Photon Systems, Inc. Whug@aol.com

Excitation-Emission Matrix Diagrams



Phenanthrene
 $C_{14}H_{10}$

Excitation
Wavelength
(nm)



Excitation (nm)	HOH	Limit
224.3	242.2	248.6
248.6	270.8	276.1

* *Bacillus anthracis* analog

Excitation-Emission Matrix Diagrams for a few target and background materials. Excitation (Ex) was from 200 to 600 nm in 5 nm increments. Emission (Em) was recorded between 200 and 600 nm. The smaller PAHs exhibit significant emission with excitation as low as 260-280 nm. As a result of their aromatic amino acid content both adenovirus and *B. subtilis* respond to excitation around 280 nm, but show significantly more activity when excited in the 200-230 nm range.

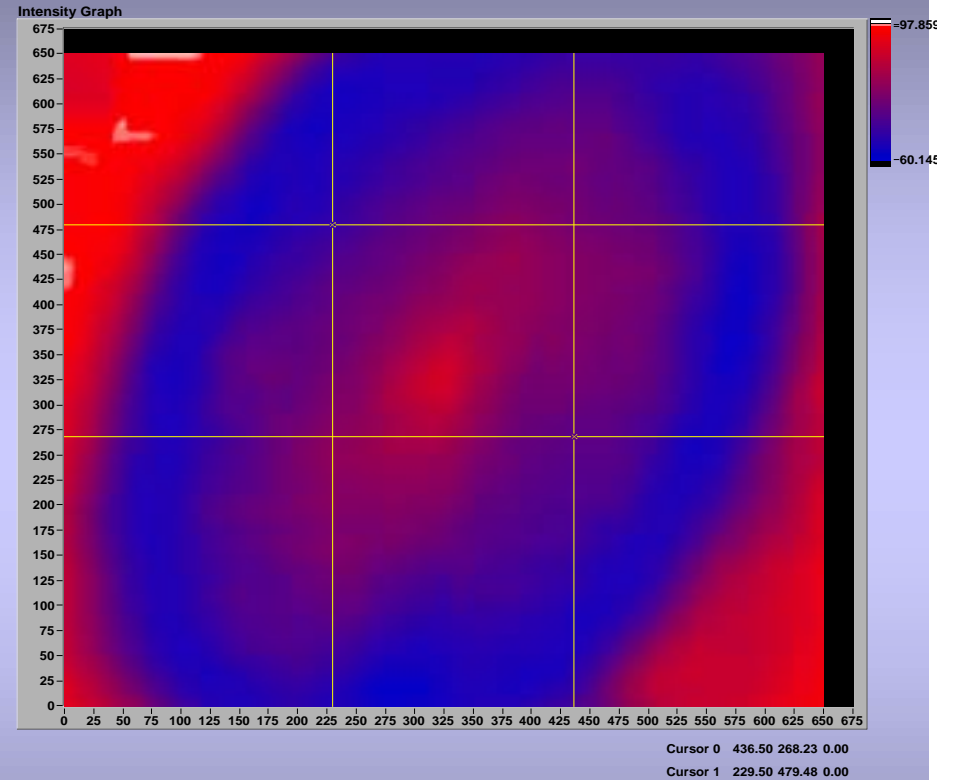
For more information contact M. C. Storrie-Lombardi, M.D. mcs1@jpl.nasa.gov

*Complexity... or whatever we decide to
call it!*

NGC-3031 and Complexity Transformation

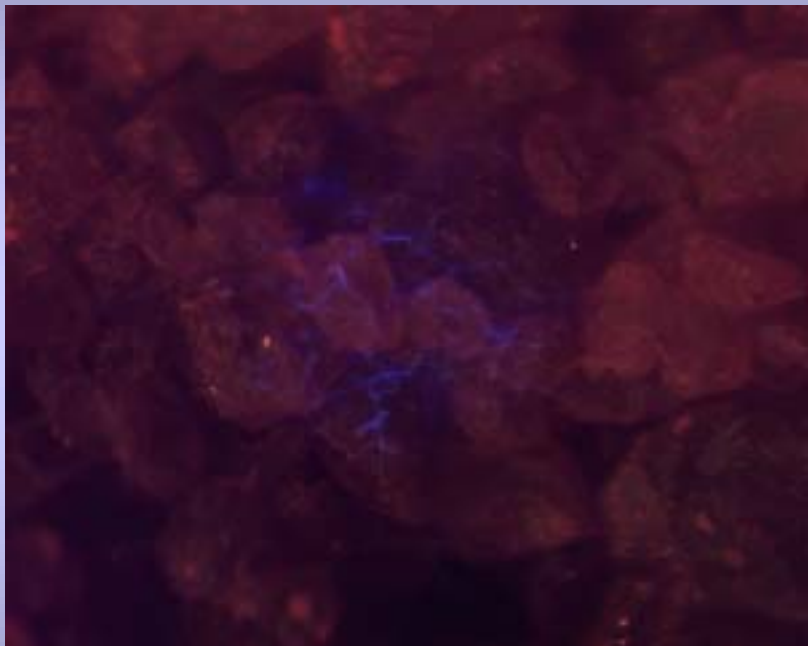


Original Image

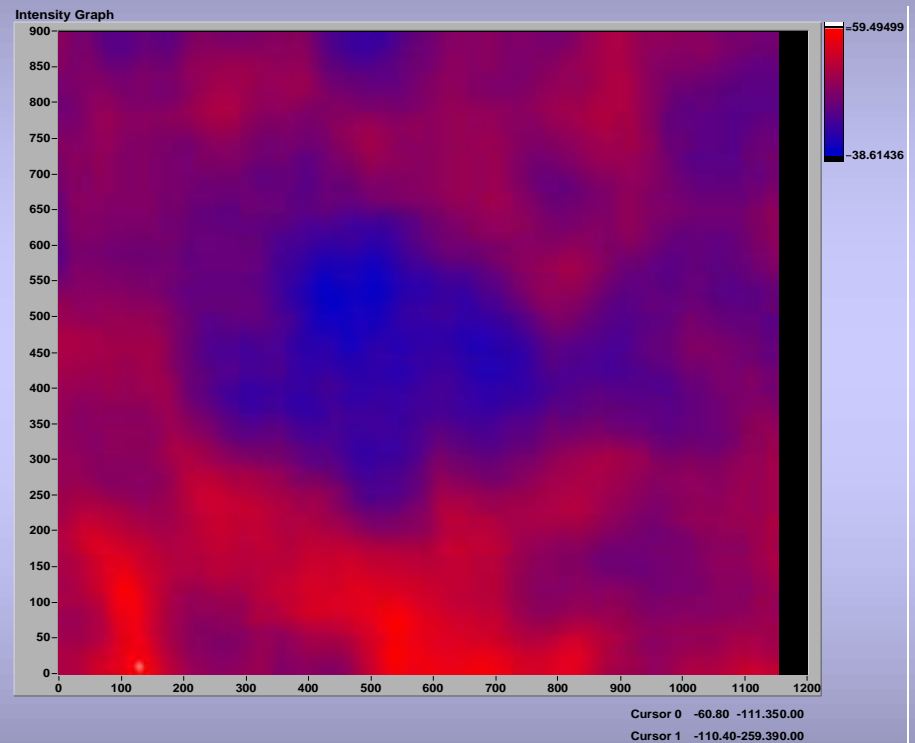


Complexity Image

Antarctic Rock – Red Zone Complexity

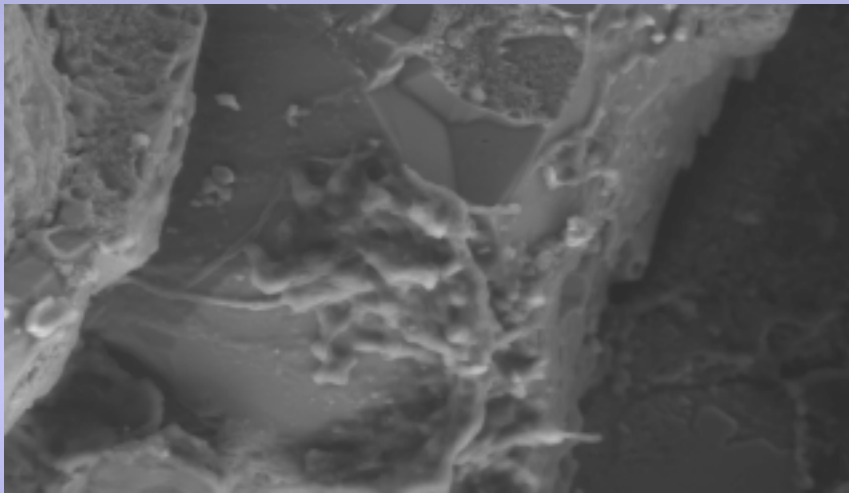


224 nm induced fluorescence

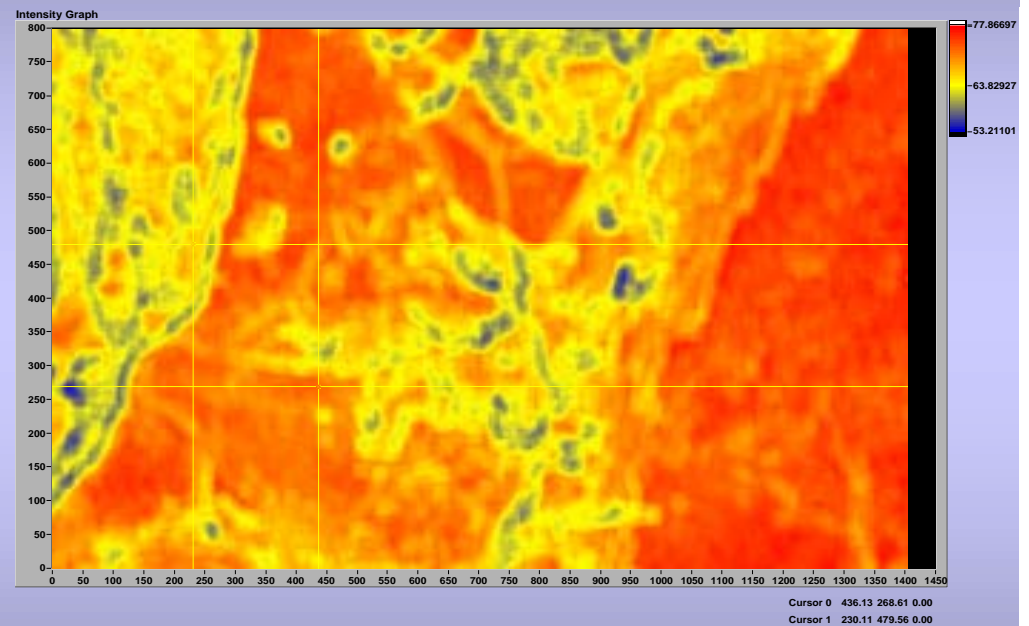


Complexity Image

Antarctic Rock – Environmental Scanning Electron Microscopy

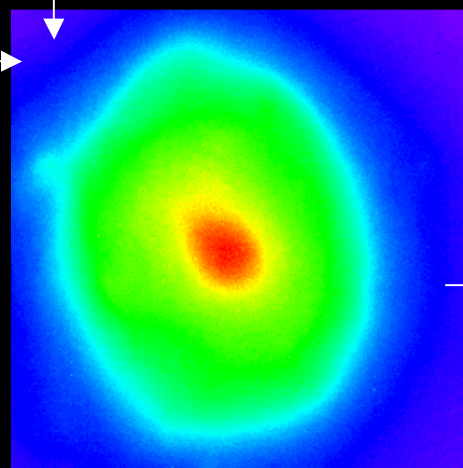
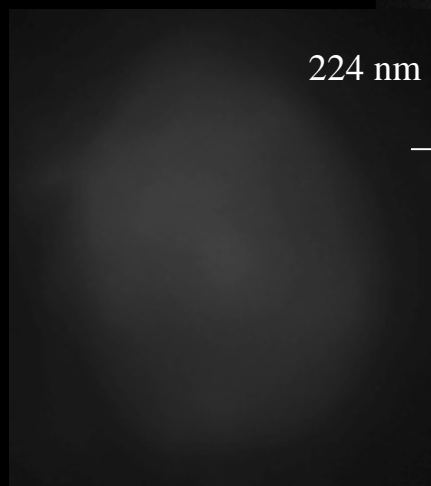
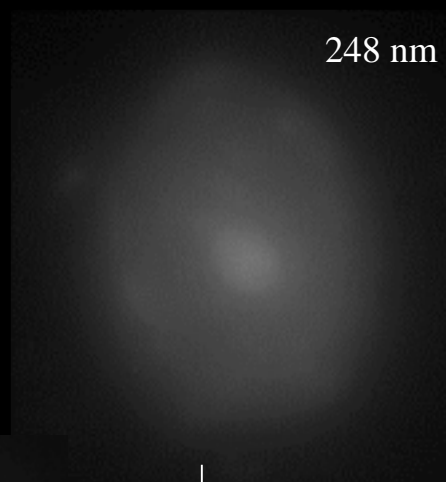
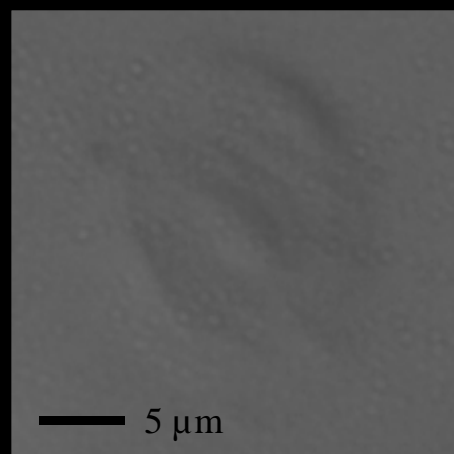


Original ESEM Image
(image courtesy of S. Douglas)

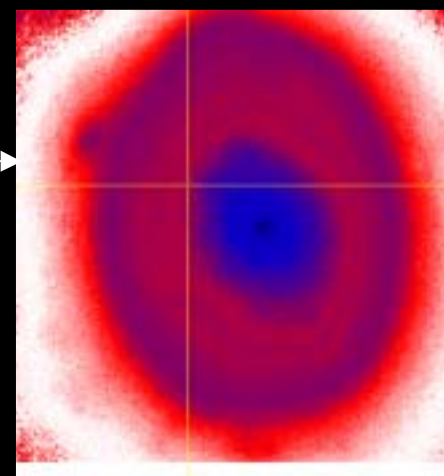


Complexity Image

Nucleus | Cytoplasm Differentiation in Human Squamous Cell



A human squamous cell exhibits native fluorescent activity after illumination at 224.3 and 248.6 nm. The resulting images were digitally subtracted. Differences in both fluorescence response and absorption of deep UV radiation by the nucleic acids and the aromatic amino acids make possible the rapid distinction of nucleus from cytoplasm. The digitally combined image was then transformed according to information content into an Entropy Image.



Stromatolites, Complexity, and Scaling:

*An example of the need for nanometer
scale data.*

Stromatolites

- Conspicuous in marine and lacustrine carbonate environs between ca. 3.5-0.544 Ga.
 - Easy to see at the outcrop scale (Rover)
- Traditional Viewpoint:
 - organosedimentary structures built by microorganisms
 - a biosignature!
- Non-traditional Viewpoint:
 - A product of diffusion limited aggregation
 - NOT a biosignature

*For more information contact M. C. Storrie-Lombardi, M.D. mcs1@jpl.nasa.gov
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Stromatolites as Biosignatures?



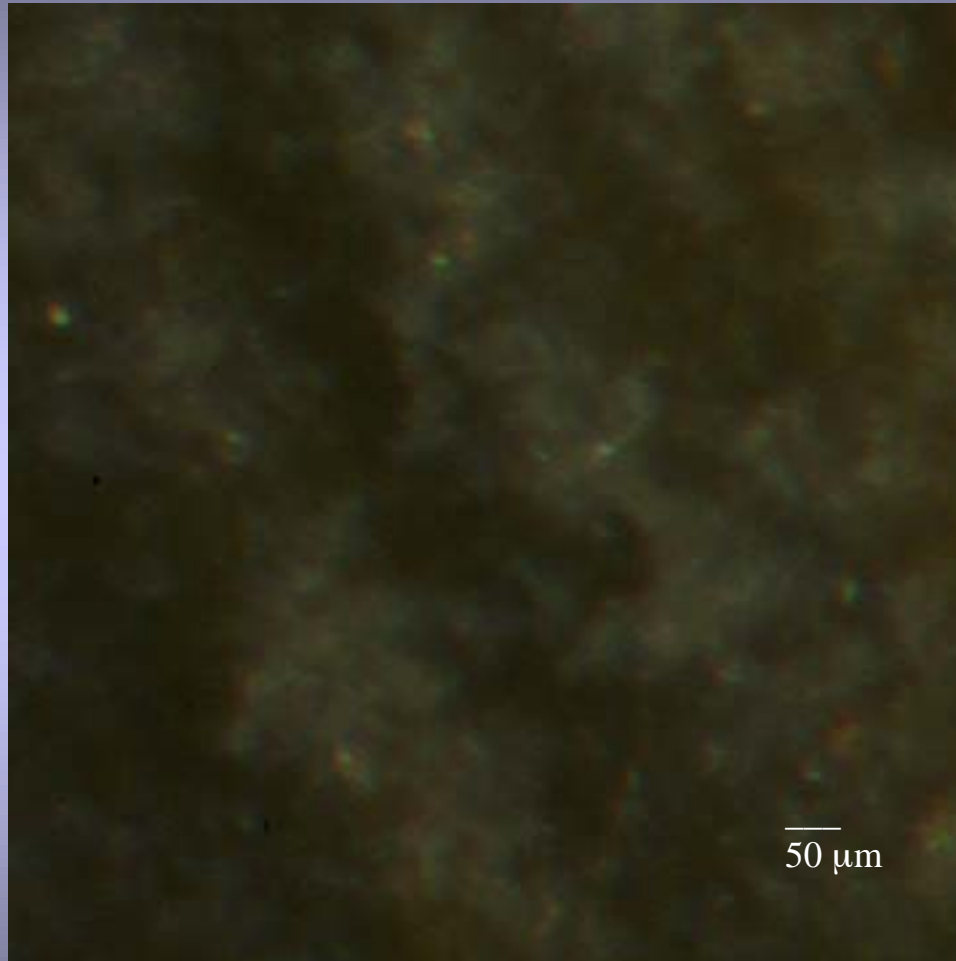
Multi-probe search for
quantitative estimates of

- Diagenesis
- Spatio-temporal shifts
- Biotic-Abiotic

Classification by
Complexity Analysis

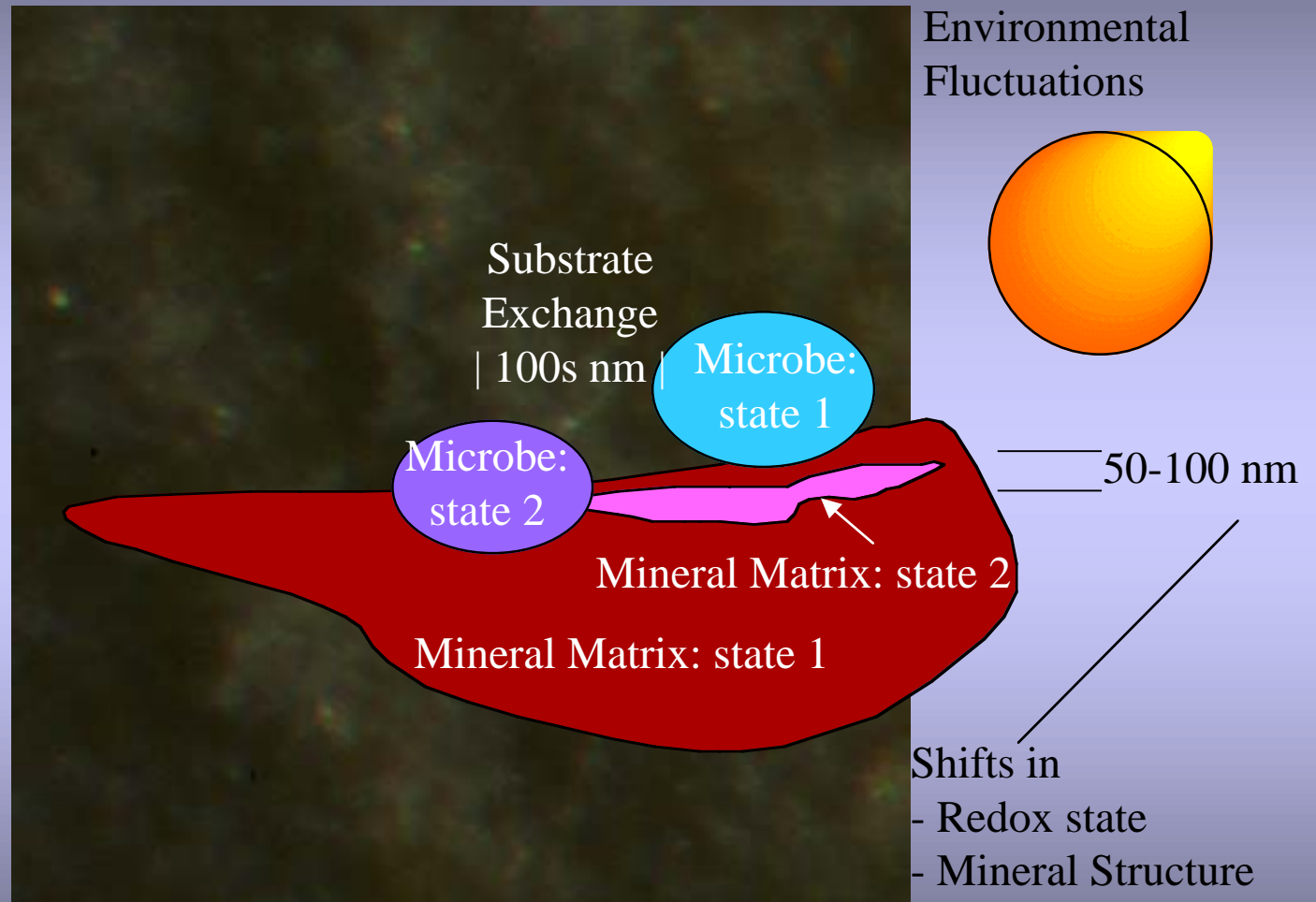
Problem: understanding
the etiology of the
patterns we are seeing
regardless of scale down
to the micrometer level.

Stromatolites as Biosignatures?

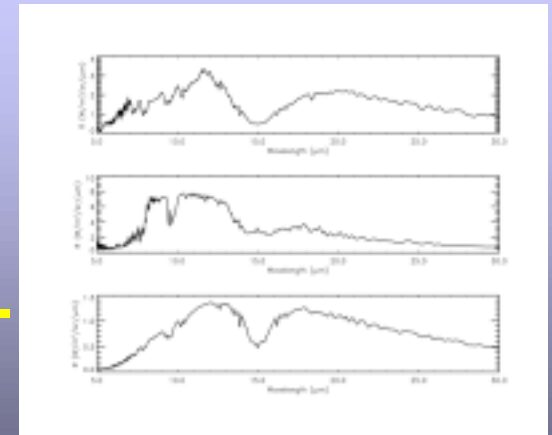
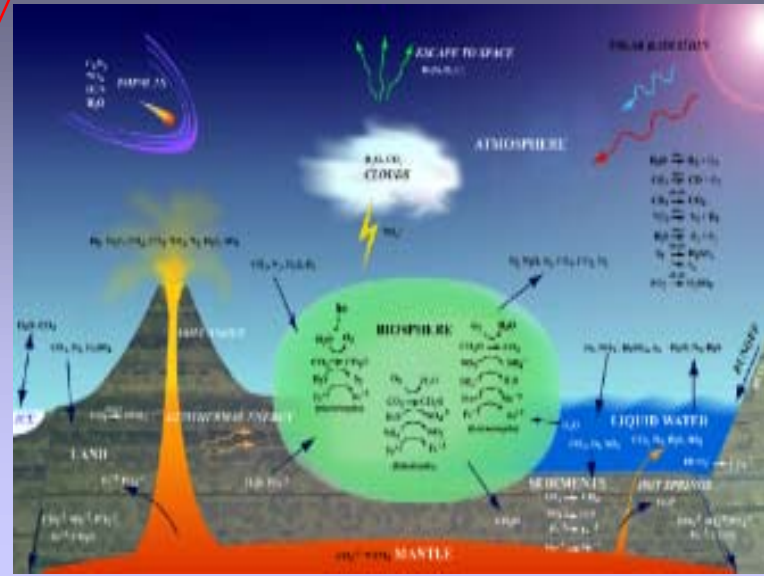
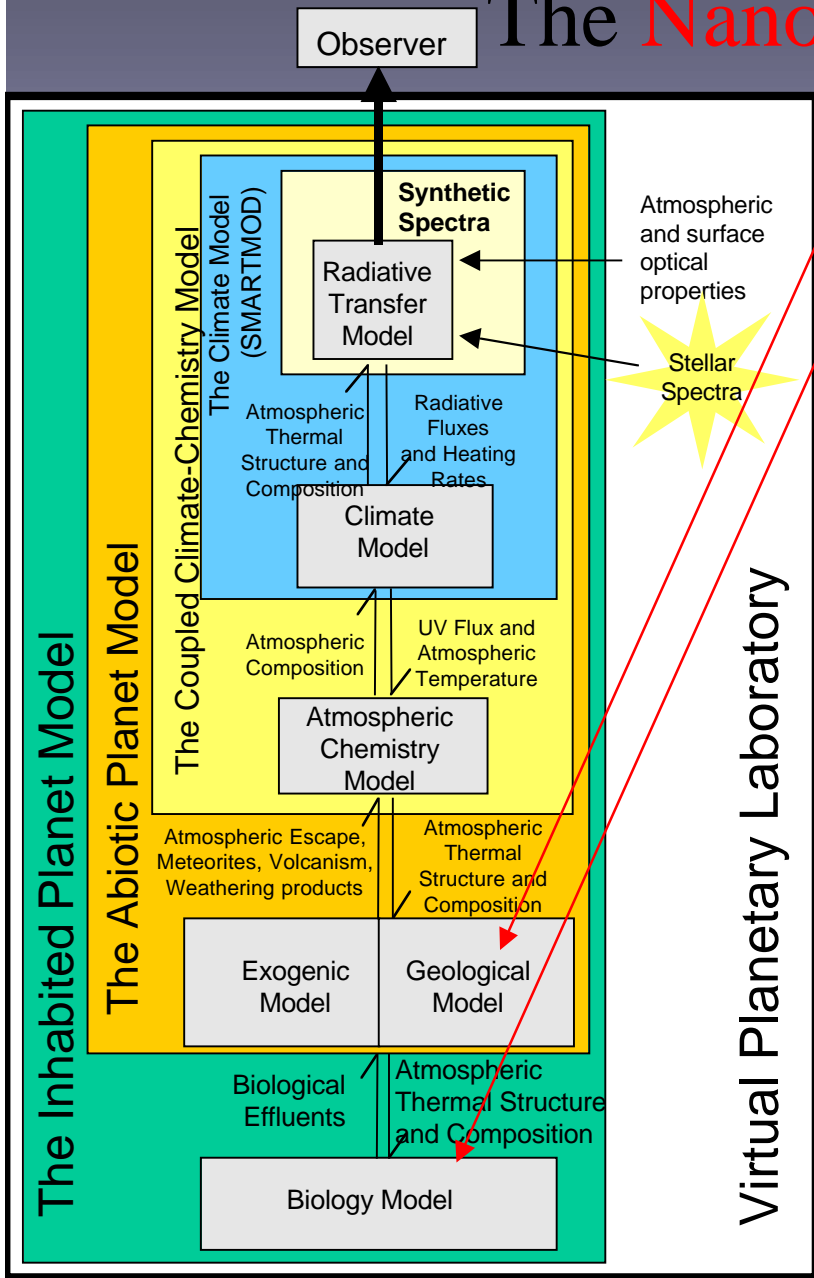


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Stromatolites as Biosignatures?

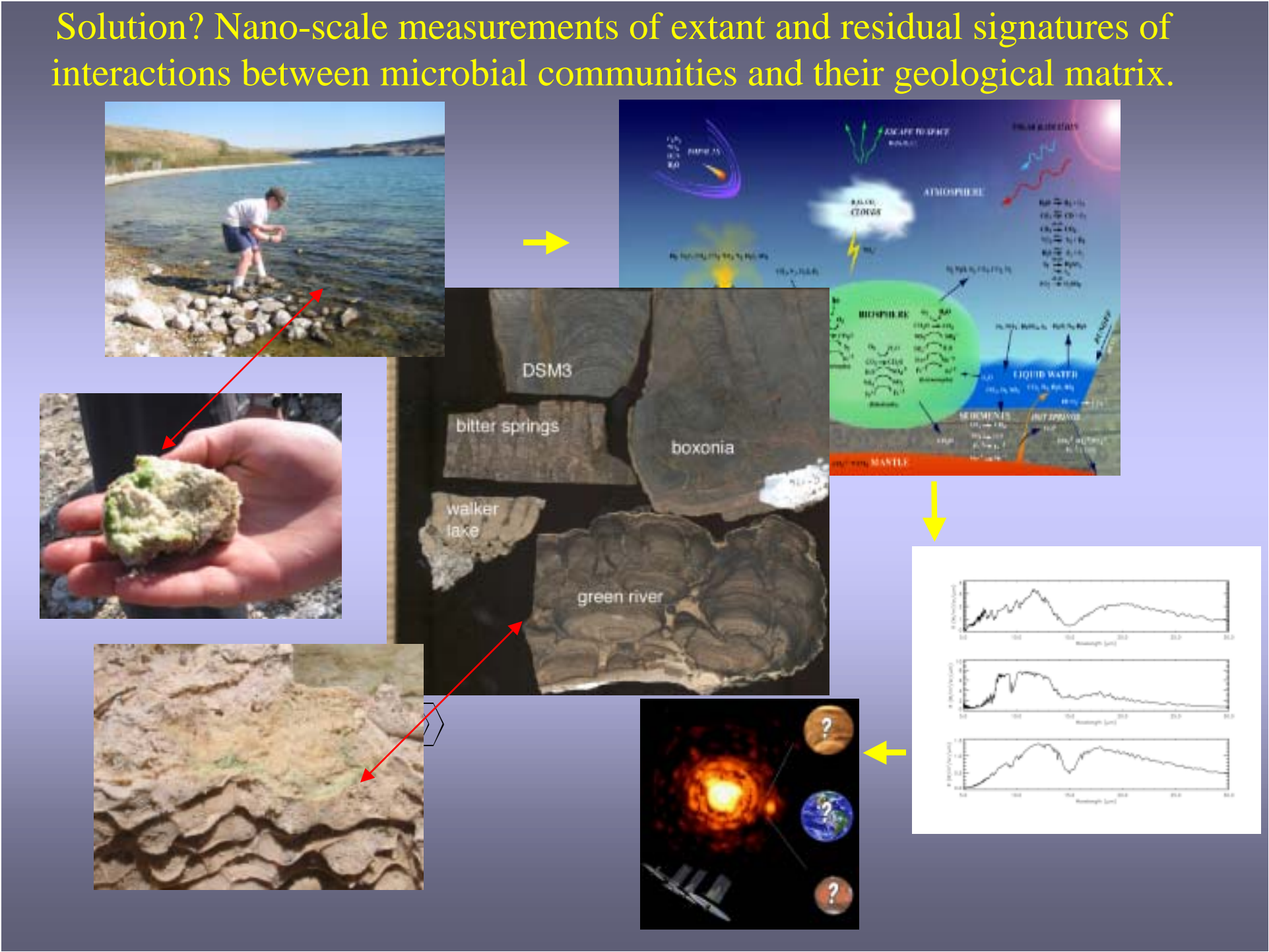
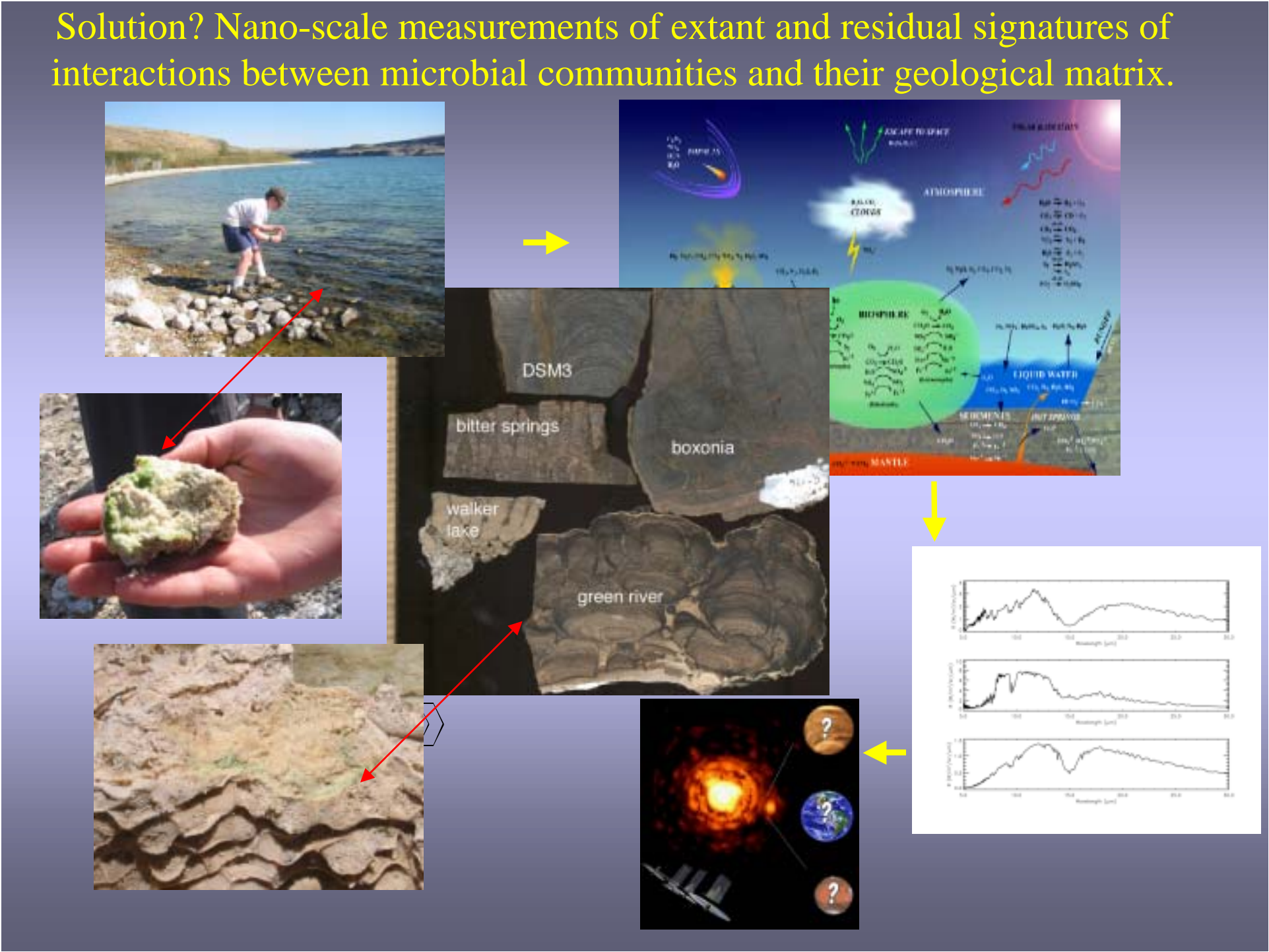
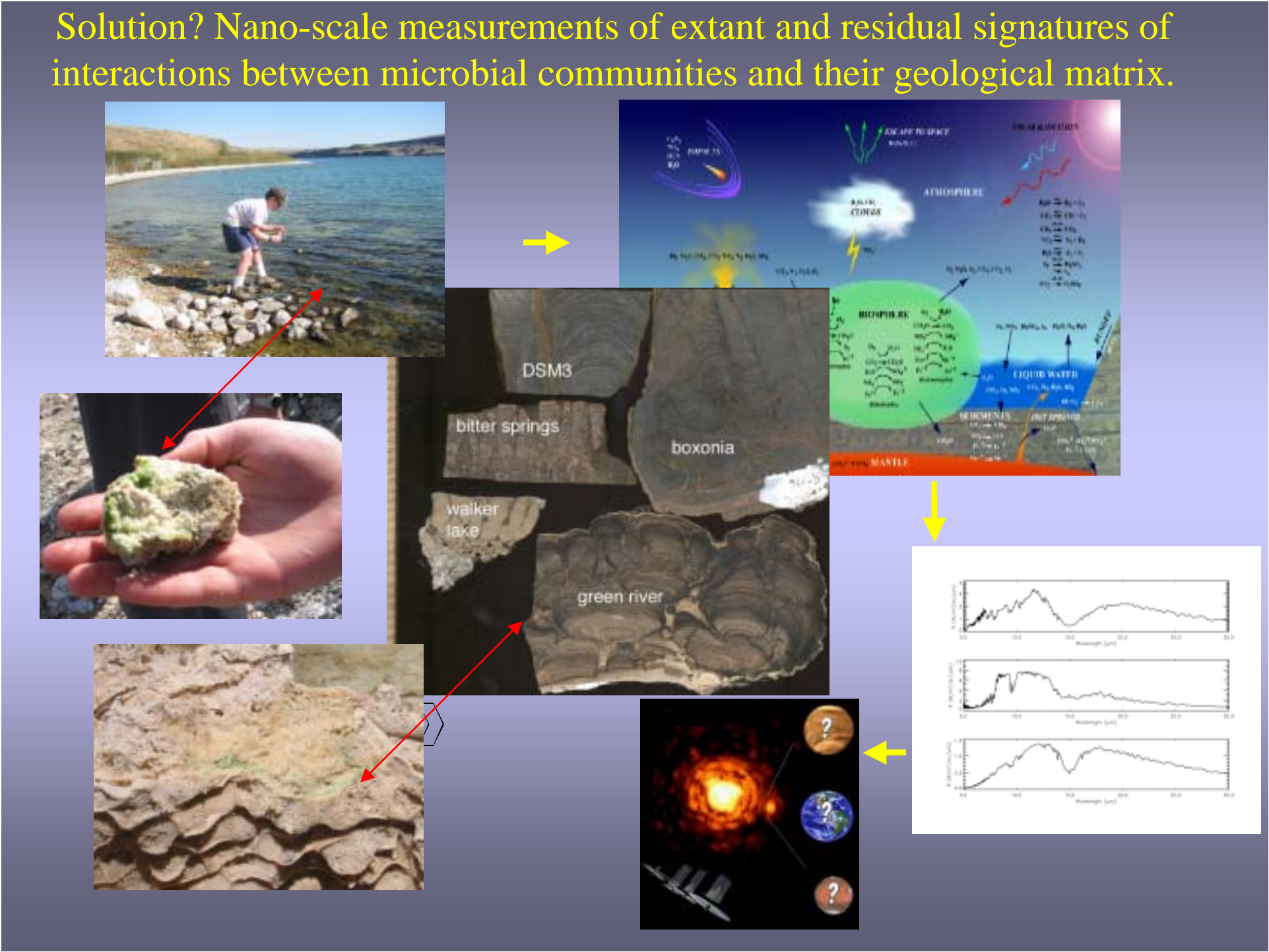
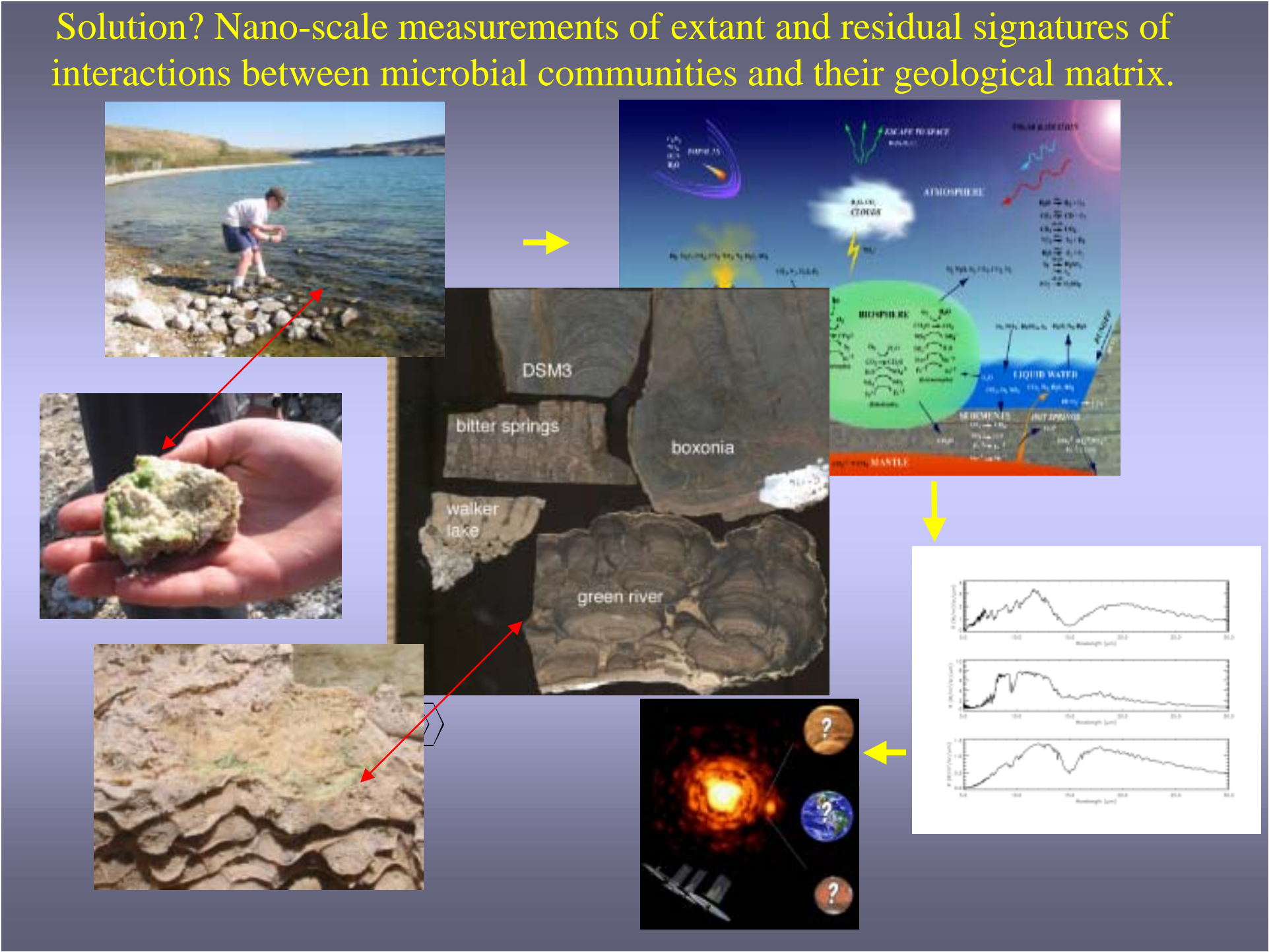
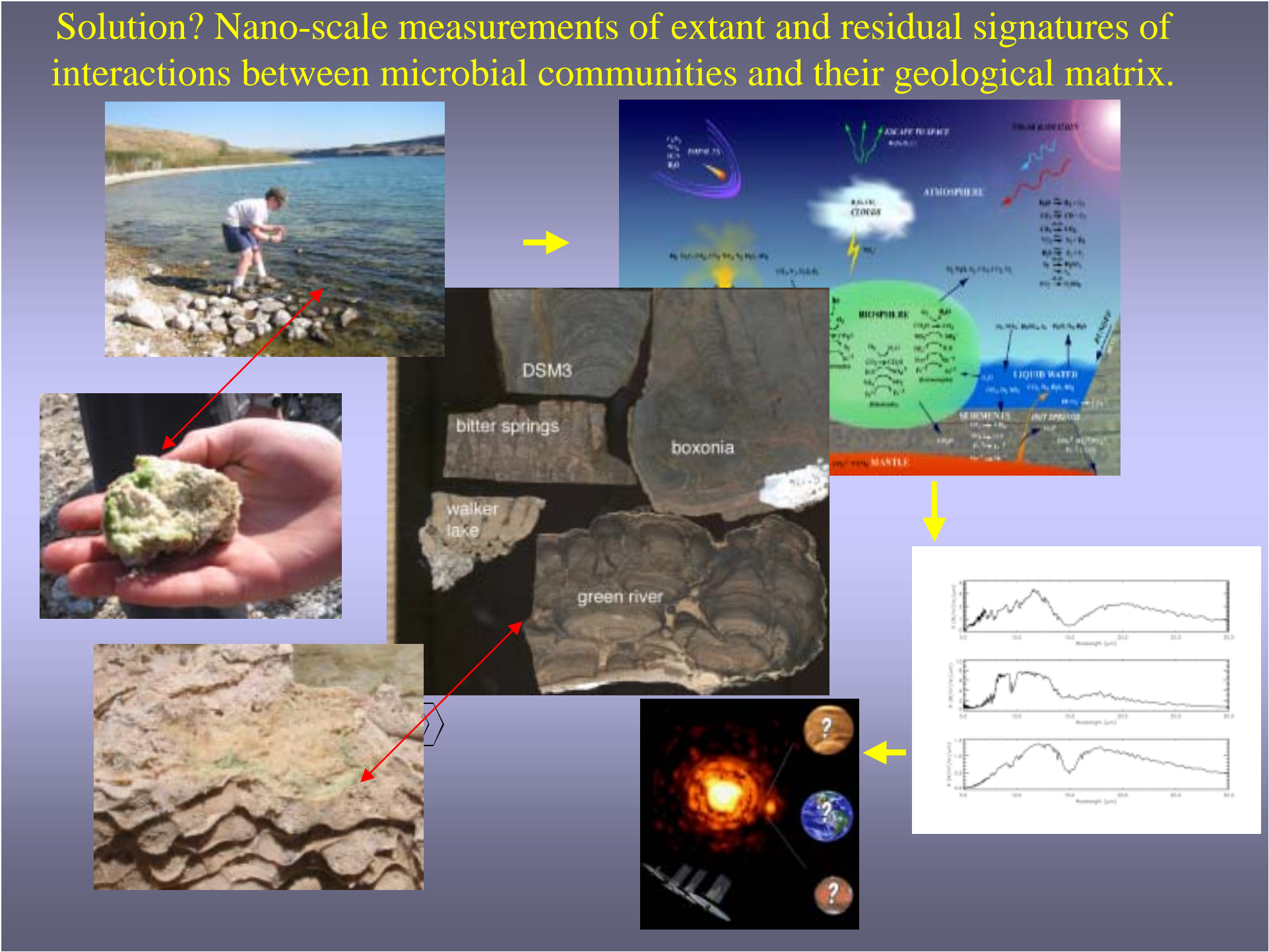
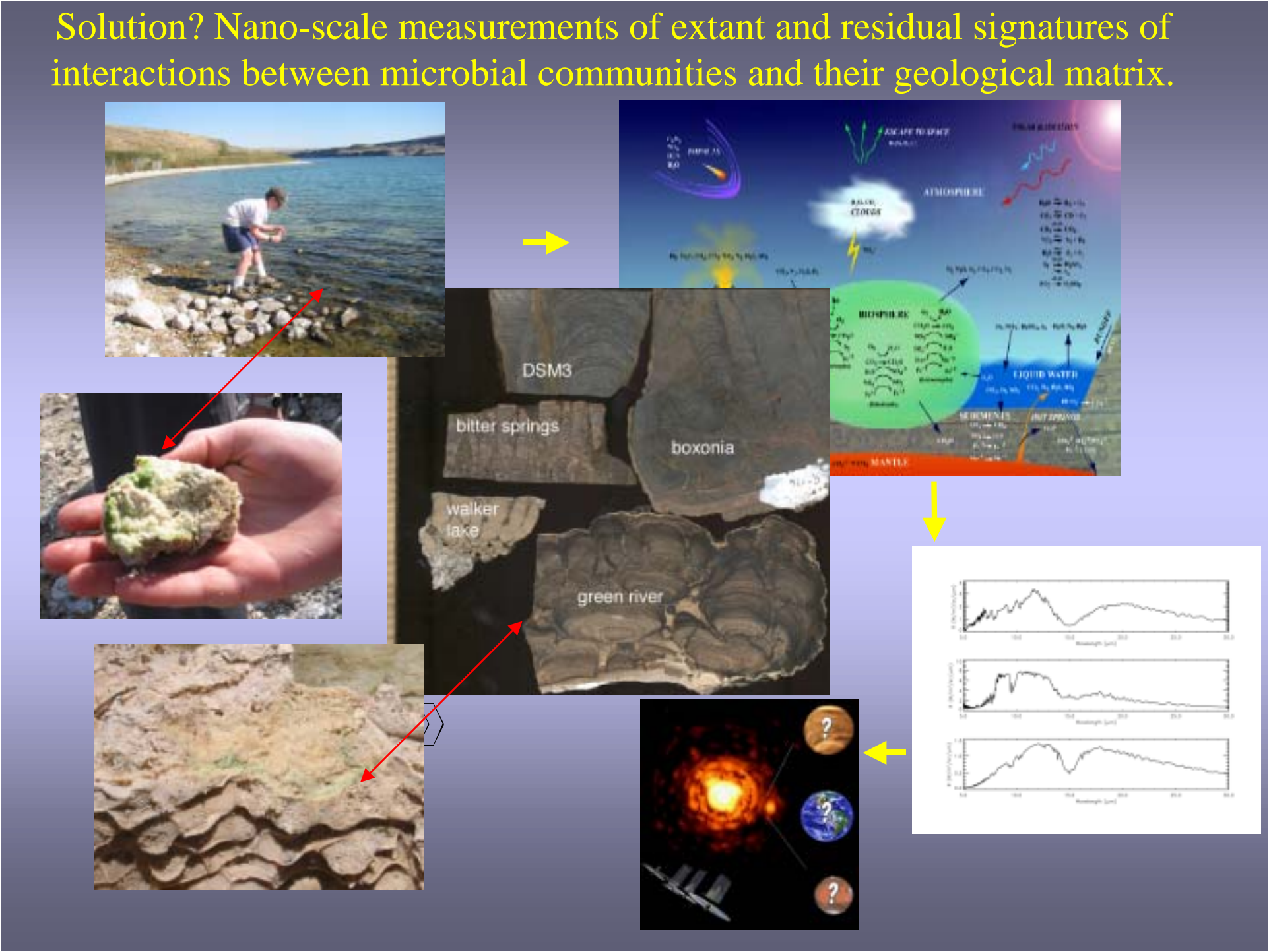
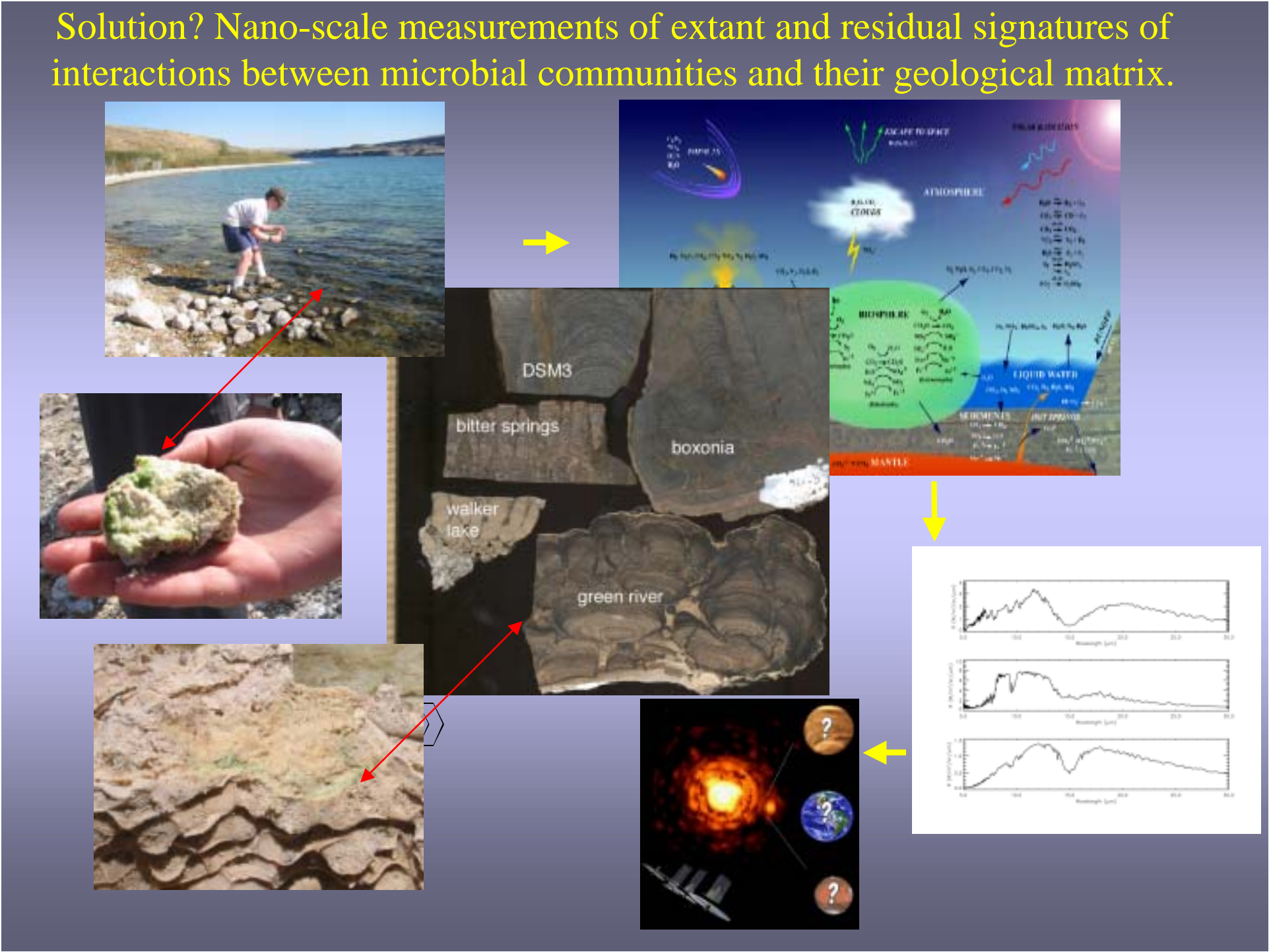


The Nano-scale Planet-finder Bottleneck



Solution? Nano-scale measurements of extant and residual signatures of interactions between microbial communities and their geological matrix.

The collage illustrates the relationship between microbial communities and geological matrices. It includes a person collecting samples from a lake, a hand holding a rock with green microbial growth, a close-up of a rock surface with microbial structures, a diagram of Earth's layers (Atmosphere, Biosphere, Liquid Water, Sediments, Mantle) with chemical signatures, and three line graphs showing isotopic data (delta 13C, delta 34S, delta 33S) versus distance. A yellow arrow points from the diagram to the graphs, and a red arrow points from the graphs to the rock surface image.



Summary Recommendations

- 1. Pursue multidisciplinary work between biology, nanotechnology and information science using the NAI distributed network as a model.*
- 2. Focus on one day providing missions with a SUITE of 20-30 instruments available for autonomous selection by a remote probe with selection dependent on real time analysis of incoming data.*
- 3. Investigate the nano-science and complexity theory constraints on building distributed survey networks of 100's to 1000's of nodes.*
- 4. Focus initially on science and technology relevant to exploration of extreme environments on this planet, and monitoring the health of both this planet and the ISS.*

“The search for life in the universe is too important to be left to adults.”

M. C. Storrie-Lombardi, M.D., 9.1.99

from an idea by A. E. Storrie-Lombardi, 10.1.96

“Dad, you’ve got 10 years, then my friends and I will find out if there is life on Mars.”